This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

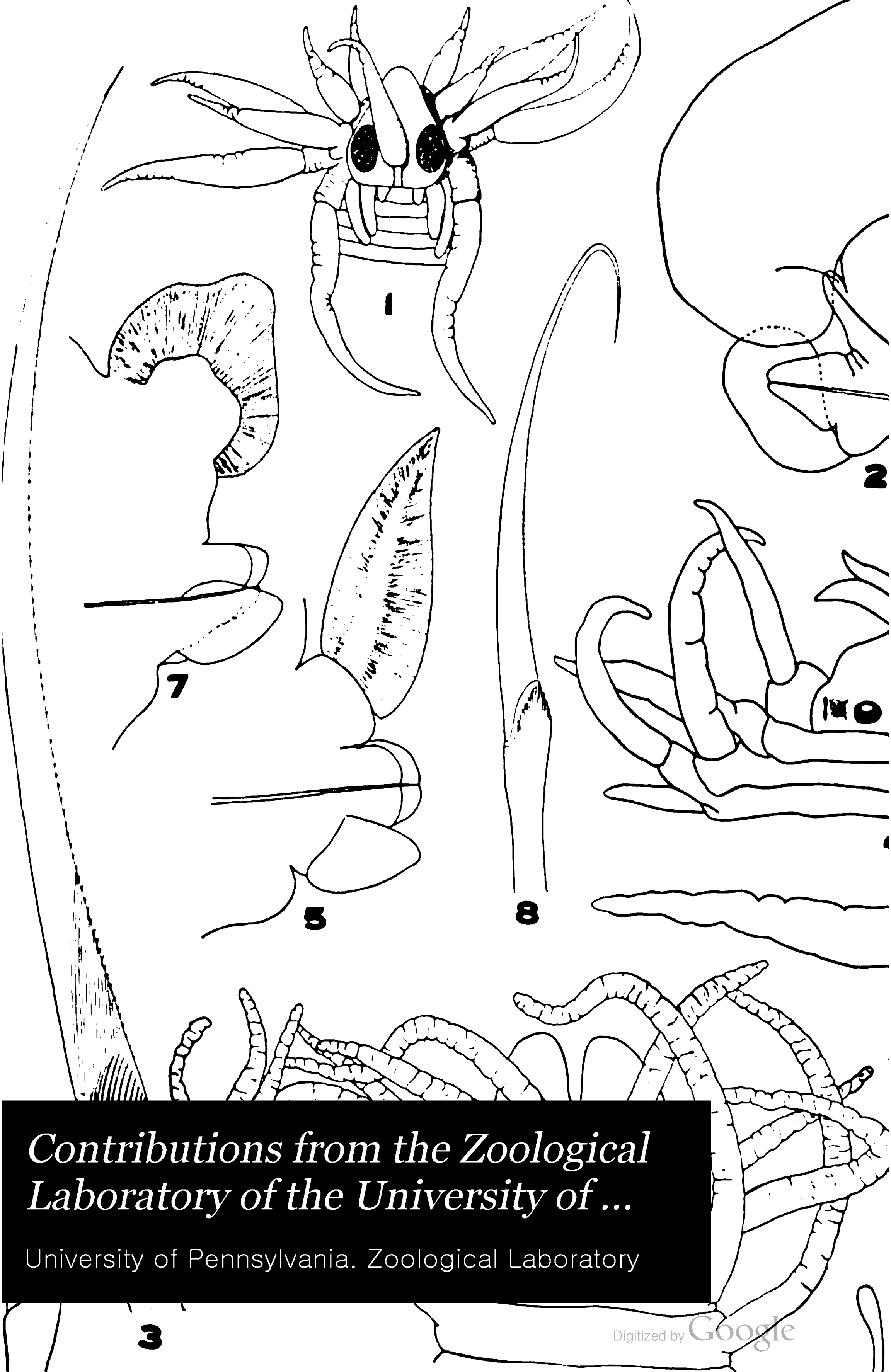
+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`



Contributions from the Zoological Laboratory of the University of ...

University of Pennsylvania. Zoological Laboratory

UNI
8186

HARVARD UNIVERSITY.

LIBRARY

OF THE

MUSEUM OF COMPARATIVE ZOÖLOGY.

13,199.

GIFT OF

Thomas H. Montgomery, Jr.

April 17, 1908.

APR 17 1908

13,199

Publications
OF THE
University of Pennsylvania

CONTRIBUTIONS
FROM THE
ZOOLOGICAL LABORATORY
FOR THE YEARS
1906-1907

VOLUME XIII
TWELVE SEPARATA

PHILADELPHIA, PA.
1908

Q.a. 65/2
35

CONTENTS

1. Conklin, Edwin G., Does Half of an Ascidian Egg give rise to a whole Larva? (Archiv für Entwicklungsmechanik der Organismen, Bd. XXI, 1906.)

2. Conklin, Edwin G., The Embryology of Fulgur: A Study of the influence of Yolk on Development. (Proceedings of the Academy of Natural Sciences of Philadelphia, July, 1907.)

4/ (3.) McClendon, Jesse F., On the Development of Parasitic Copepods. (Biological Bulletin, Vol. XII, 1906-1907.)

3 (4.) McClendon, Jesse F., Notes on the true Neuroptera. (Entomological News, Vol. XVII, April, 1906.)

5. Moore, J. Percy, New Species of Ampharetidae and and Terebellidae from the North Pacific. (Proceedings of the Academy of Natural Sciences of Philadelphia, December, 1905.)

5 (6.) Moore, J. Percy, Description of two new Polychaeta from Alaska. (Proceedings of the Academy of Natural Sciences of Philadelphia, July, 1906.)

6 (7.) Moore, J. Percy, Additional new Species of Polychaeta from the North Pacific. (Proceedings of the Academy of Natural Sciences of Philadelphia, April, 1906.)

8. Moore, J. Percy, Descriptions of new Species of Polychaeta from the Southeastern Coast of Massachusetts. (Proceedings of the Academy of Natural Sciences of Philadelphia, November, 1906.)

9. **Moore, J. Percy**, Descriptions of new Species of Spioniform Annelids. (Proceedings of the Academy of Natural Sciences of Philadelphia, May, 1907.)

10. **Pearl, Raymond**, Variation in the Number of Seeds of the Lotus. (American Naturalist, Vol. XL, November, 1906.)

11. **Steele, Mary Isabelle**, Regeneration in Compound Eyes of Crustacea. (Journal of Experimental Zoology, Vol. V, December, 1907.)

12. **Surface, Frank M.** The Formation of new Colonies of the Rotifer, Megalotrocha alboflavicans, Ehr. (Biological Bulletin, Vol XI, September, 1906).

Does Half of an Ascidian Egg give rise to a Whole Larva?

By

Edwin G. Conklin,
University of Pennsylvania, Philadelphia.

With 32 figures in text.

Eingegangen am 9. April 1906.

In one of the earliest experimental studies on the potency of individual cleavage cells, CHABRY (1887) found that surviving blastomeres of the egg of *Ascidia aspersa* gave rise only to partial larvae and he summarizes his extensive and detailed work as follows (p. 289): »De là on tire aisément la conclusion (que je ne crois valable que pour l'Ascidie et les animaux, dont les blastomères sont différenciés de bonne heure), que chaque blastomère contient en puissance certaines parties dont sa mort entraîne la perte irrémédiable et que les différentes parties de l'animal sont préformées dans les différentes parties de l'oeuf.«

These conclusions were discussed by several authors, among whom DRIESCH (1892) and O. HERTWIG (1892) concluded from CHABRY's own figures that the fractional embryos and larvae obtained by him were really entire and not partial; whereas ROUX (1892), WEISMANN (1892) and BARFURTH (1893) held the opposite view. Afterwards DRIESCH (1895) confirmed his opinion by experiments on the eggs of *Phallusia mammilata.* He found that the cleavage was neither »halb« nor »ganz« but »regellos solid«; that the gastrulae, though small, where otherwise entirely like those of normal eggs; and that whole larvae were formed from any one or more of the first four blastomeres. He concluded, in short, that the development of isolated blastomeres of the ascidian egg was like that of the echinoderm: »Aus isoliert überlebenden Blastomeren des Ascidieneies entwickelt sich nicht ein halber (bzw. viertel, dreiviertel), rechter oder linker

(bzw. vorderer oder hinterer) Embryo, sondern stets ein ganzer von halber Größe, dem allerdings (meist) gewisse Organe von minderer Bedeutung (Otolith, ein Haftorgan) fehlen« (p. 405).

Subsequently (1903) Driesch found that if the cup-shaped gastrulae of *Phallusia* were cut in two transversely each half gave rise to a normal larva, whereas when the elongated gastrulae were cut in two in the same manner, the anterior half gave rise only to a head and the posterior half to a tail.

Crampton (1897) also studied the potency of individual cleavage cells of Ascidians and found that an isolated blastomere of the egg of *Molgula manhattensis* segments as if still forming a part of an entire embryo; »the cleavage phenomena are strictly partial as regards the origin of cells, the inclination of cleavage planes, and especially in respect to the rhythm of segmentation«. He did not observe the gastrulation but says that »a larva arises which resembles the normal larva, except as regards smaller size and certain minor defects« (p. 55).

In the summer of 1904 I repeated the experiments of Chabry, Driesch and Crampton on the eggs of *Cynthia* (*Styela*) *partita* and *Molgula manhattensis* and, for these froms at least, obtained what I believe to be convincing evidence of the accuracy of Chabry's results as opposed to those of Driesch. Each isolated and uninjured blastomere continues to segment as if it were still part of an entire egg; a half gastrula (right or left, anterior or posterior) is formed composed of identically the same cells and which occupy almost the same positions, as in the corresponding half of a normal gastrula; and finally a partial larva is formed which lacks entirely those organs which typically would have developed from the injured blastomere. In right or left half larvae, the muscle cells, mesenchyme and probably the atrial invagination and papilla (Chabry) of one side are wholly lacking; anterior $^2/_4$ larvae have no muscle cells whatever and never develop a tail; posterior $^2/_4$ larvae have neither nervous system, notochord nor gastral endoderm and show no traces of a head or trunk. Regulation is limited entirely to the closure of the larva (or organ) upon the injured side and there is no restitution of missing parts. Finally if early or late gastrulae be cut in two transversely nothing even remotely resembling a normal larva is ever produceed, (Conklin 1905[3]).

Since these results conflict in every particular with those of Driesch and have recently (1905) been called in question by him it may be fitting for me to state that my experimental work was undertaken only after I had made a thorough study of the normal development of these ascidians, in which I traced the lineage of every cell as far as the gastrulation (218 cell-stage), while the cellular origin of all the principal organs of the larva was definitely determined. Furthermore the eggs and embryos of *Cynthia* are peculiarly favorable for experimental work since four or five distinct ooplasmic substances (ectoplasm, endoplasm, myoplasm, chymoplasm, and chorda-neuroplasm) which enter into the formation of the different germ layers and chief organs of the larva are differently colored and may be clearly recognized, and even photographed (CONKLIN 1905[2]) in the living egg. Finally my studies of fractional larvae were made not merely upon living specimens but in large part upon fixed and stained material, either mounted entire in such manner as to show plainly every cell of the larva, or prepared as serial sections. I have carefully studied several hundred of these partial larvae and in support of my conclusions have published 70 camera drawings of partial embryos in almost every stage of development, (CONKLIN, 1905[3]).

It seems to me worth while to emphasize these details regarding methods and material, since with methods of observation no more modern nor exact than those known to the earliest users of the microscope I might have reached different conclusions.

My work has now been assailed by DRIESCH (1905) in his general review on »Entwicklungsphysiologie«. This attack is based not upon a new investigation of the subject, but, like his first criticism of CHABRY's work, upon a study of the paper in question in the light of his preconcieved theories. Apart from general charges against me of ignorance, begging the question, etc., DRIESCH's criticism is largely limited to my account of the development of one of the first two blastomeres. In short he maintains that the larvae obtained by me from one of the first two blastomeres were really entire (or approximately such), and that his former conclusions are therefore supported by my work.

1. Cleavage.

Regarding the cleavage of $^1/_2$ blastomeres DRIESCH says: »CONKLIN findet bezüglich der Gesamtkonfiguration des $^1/_2$-Furchungsbildes dasselbe wie ich.« Specifically, DRIESCH (1895) found in the cleavage of the $^1/_2$ blastomere of *Phallusia* that micromeres are formed in some cases, in others not; that there is no correspondence in the position of cells derived from the $^1/_2$ blastomere and those constituting the right or left halves of an entire egg; that in every case the $^1/_2$ or $^1/_4$ blastomere gives rise to a solid mass of cells; and finally that the cleavage is neither »halb« nor »ganz«, but »regellos solid«.

On the other hand I found as did CHABRY and CRAMPTON, that the cleavage of an uninjured, isolated blastomere is the same as if that blastomere were still part of the entire egg, save for very slight changes in the direction of division in some of the cells. In the rhythm of cleavage; the size, histological character and lineage of every cell; the isolation of particular ooplasmic substances in particular cells the cleavage of a $^1/_2$ blastomere is like that of the corresponding half a normal egg. In *Cynthia* and *Molgula* such cleavage forms are never bilaterally symmetrical; and they are no more solid and no more irregular or inconstant than are those of the normal egg, provided the surviving blastomere is uninjured.

In support of this statement I refer to Figs. 13—26 of my former paper in which the lineage of every cell derived from a $^1/_2$ blastomere is given as far as the the 64-cell stage (32 cells in the $^1/_2$ embryo), while in other cases I have traced the lineage of every cell as far as the 112-cell stage. I have recently reviewed many of my stained and mounted preparations of these stages and I find the most abundant and detailed confirmation of these conclusions.

2. Gastrula.

Regarding the character of the $^1/_2$ gastrula, DRIESCH says that the single figure which I give of the invagination stage (Fig. 31) is wholly insufficient to demonstrate its half character. This figure is a detailed and accurate camera drawing; it shows the gastrula open along the injured side; the cells which contain the different ooplasmic substances (ectoplasm, endoplasm, chorda-neuroplasm, myoplasm, chymoplasm) are present as in the right half of a typical gastrula; even in general form as well as in the number and character of the individual cells this gastrula is like the half of a typical one and I had supposed that one such figure would be sufficient. However since this is not the case I gladly furnish additional figures¹).

---

¹) All figures in this paper are camera lucida drawings at stage level of embryos or larvae of *Cynthia* (*Styela*) *partita*, seen with ZEISS Achromatic

Figures 1—4 represent different aspects and stages of $^1/_2$ gastrulae of *Cynthia*, and it can be seen at a glance that the muscle, mesen-

Fig. 1.

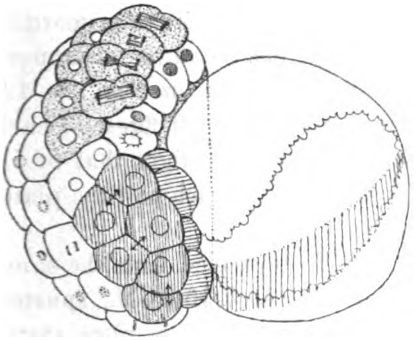


Fig. 2.

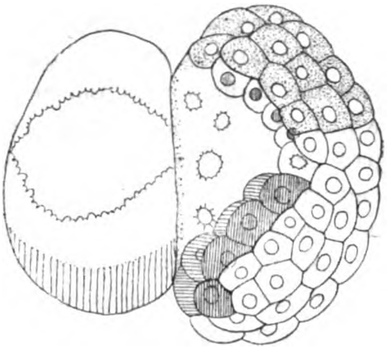


Fig. 3.

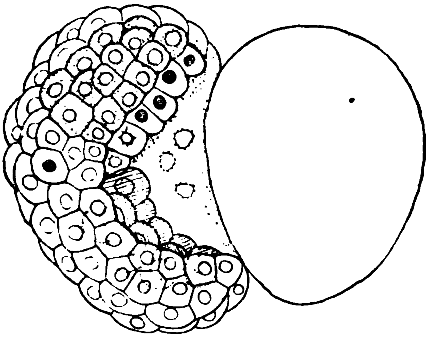
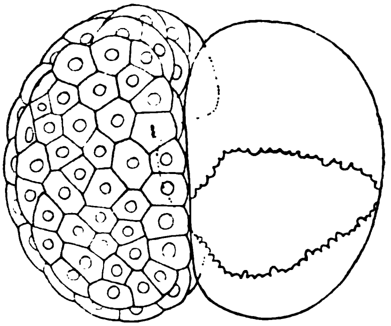


Fig. 4.



Figs. 1—4. Half-Gastrulae of *Cynthia*, stained and mounted as entire preparations. Figs. 1—3 dorsal views, with blastopore more or less widely open; Fig. 4, ventral view. Muscle and mesenchyme cells (shaded by vertical and by transverse lines) lie along the lateral-posterior border of the blastopore; neural plate and chorda (indicated by stippled cells and by shaded nuclei respectively) lie along the anterior border; the crescent of mesoplasm in the injured blastomere is shown by vertical shading in Figs. 1 and 2.

Fig. 1 corresponds almost cell for cell with the left half of a normal gastrula (see Fig. 8 of my former paper); four mesenchyme cells are partially invaginated, while six muscle cells lie at the surface; eight neural plate cells are present in two rows, one of which is dividing and there are four chorda cells in a single row, exactly as in the half of a normal gastrula, but the rows of neural plate and chorda cells are here more antero-posterior in direction than in a typical form. The exact lineage of each of these cells may be determined by comparing this figure with Fig. 8 of my previous paper.

Figs. 2 and 3 show later stages of the infolding of the mesenchyme and muscle cells; in these figures the identity of some of the neural plate cells is somewhat doubtful, but these gastrulae are plainly half-structures.

Fig. 4 shows only the ectoderm cells of the ventral surface; the outline of the half-blastopore, seen through the embryo, is shown in dotted line.

Immersion Obj. $^1/_{12}$, Oc. 4, and therefore magnified about 850 diameters. In the process of reproduction the figures have been reduced one-third, so that as chyme, neural plate and chorda cells are present in the same relative numbers and in almost the same positions as in the corresponding half of a typical gastrula. The ectoderm cells have grown in along the injured side to a slight extent, but there is no change in the axes of the gastrula, nor in the general position of its organ primordia.

The later gastrulae of *Cynthia*, up to the closure of the blastopore and the formation of the neural groove, are shown in Figs. 5—12, which are camera drawings of actual sections, the left figure of each pair being that of a normal, uninjured embryo, the right that of $^1/_2$ embryo of corresponding stage; in both figures of each pair the plane of section is approximately the same.

All the embryos shown in Figs. 5—12 developed from the same lot of eggs; these eggs were laid normally at 5:15 P. M., spurted in the two-cell stage at 6:15 P. M., and then fixed in picro-acetic acid at 9:45 P. M.; all these embryos are therefore of the same age and they show only slight differences in extent of development. It will be understood, of course, that in the spurting some of the eggs remained uninjured and gave rise to normal embryos, others had one of the first two blastomeres killed and gave rise to $^1/_2$ embryos, while in still other cases both blastomeres were killed.

Figures 5—8 are frontal-longitudinal sections of gastrulae with small posteriorly placed blastopore; Fig. 5 is from near the ventral side and shows within the one-layered ectoderm the darkly staining crescent of muscle and mesenchyme cells and anterior to these the yolk-laden endoderm cells; Fig. 6 is a corresponding section of a $^1/_2$ embryo showing muscle, mesenchyme and endoderm cells of one side only, and in approximately the same positions as in the left half of Fig. 5; in this $^1/_2$ embryo the ectoderm has begun to grow in along the injured side, especially at the anterior end.

Fig. 7 is a frontal-longitudinal section near the dorsal side, showing muscle cells bounding the blastopore laterally, while chorda and endoderm cells lie anterior to the blastopore, the chorda cells

---

they now stand they are magnified about 567 diameters. In all the figures the muscle cells are shaded with vertical lines, the mesenchyme with transverse lines, while the neural plate and tube and are stippled; the nuclei of the chorda cells are shaded by lines, and in the endoderm cells the boundary between the cytoplasm and yolk is marked by a crenated line. In the injured blastomeres the boundary between cytoplasm and yolk is also indicated by a crenated line and the crescent of mesoplasm (myoplasm und chymoplasm) is shaded by vertical lines. Material was fixed chiefly in Picro-Acetic, stained in Delafield's Haematoxylin, and either mounted entire (Figs. 1—4) or cut into serial sections.

being in process of division. In Fig. 8 the plane of section is not exactly the same as in Fig. 7, the anterior part of the section being

Fig. 5.

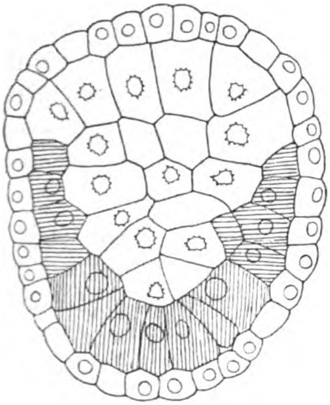


Fig. 6.

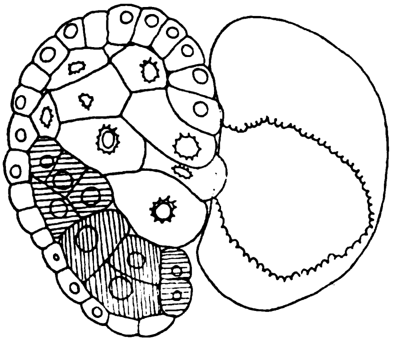


Fig. 7.

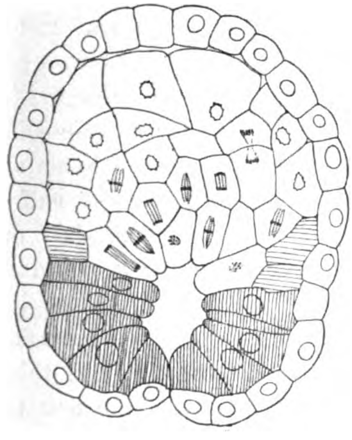
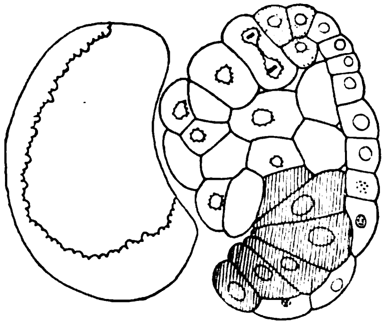


Fig. 8.



Figs. 5—8. Frontal-longitudinal sections of slightly elongated gastrulae from Exp. 14 (Laid 5:15 P. M.; Spurted 6:15 P.M.; Fixed 9:45 P. M.). Figs. 5 and 7, normal gastrulae from uninjured eggs; Figs. 6 and 8, half-gastrulae from eggs in which one of the first two blastomeres was injured.

Figs. 5 and 6. Sections near the ventral side; in Fig. 5 the muscle and mesenchyme cells, as a deep staining crescent, surround the endoderm on the posterior side; in Fig. 6 ectoderm has grown in to a slight extent along the anterior border of the injured side.

Figs. 7 and 8. Sections near the dorsal side, the anterior end of Fig. 8 being sufficiently near the dorsal surface to take in some of the neural plate cells (stippled). In Fig. 7 the blastopore is bounded laterally by muscle cells and anteriorly by chorda cells, which are here dividing. In Fig. 8 the neural plate and the muscle cells of the right side only are present, chorda and endoderm cells are not distinguishable; ectoderm has not overgrown the injured side.

nearer the dorsal side and the posterior, nearer the ventral; the muscle cells are in approximately the same position as in the right half of Fig. 7, but it is here difficult to distinguish between the chorda and endoderm cells; in this figure the ectoderm has not grown in along the injured side; there are three deeply staining cells at the anterior edge of the ectoderm which probably represent the half of the neural plate.

Figures 9—12 are transverse sections of embryos similar to the preceding. Fig. 9 is a section through the open blastopore in the posterior third of the embryo; on each side of the blastopore and gastrocoel are the large muscle cells, at the bottom of the gastrocoel is the double row of caudal endoderm cells, while on each side of the latter is a group of smaller mesenchyme cells. Fig. 10 is a corresponding section of a $^1/_2$ embryo in approximately the same plane and position as Fig. 9; the muscle, mesenchyme and caudal endoderm cells occupy almost exactly the same positions as in the right half of Fig. 9, but the ectoderm has grown in along the injured side to a limited extent.

Figures 11 and 12 are slightly more advanced embryos, though from the same lot of eggs as the preceding; both represent transverse sections in front of the blastopore and through the neural groove. In the normal embryo (Fig. 11), the endoderm and chorda cells surround the gastric cavity, a group of mesenchyme cells lies on each side, while dorsally is the neural groove; in the corresponding section of the $^1/_2$ embryo shown in Fig. 12, the mesenchyme and neural groove are present almost exactly as in the right half of Fig. 11, but there is no gastric cavity and I cannot certainly distinguish chorda and endoderm cells; the ectoderm has not overgrown the endoderm along the injured side.

These sections, taken in conjunction with the entire preparations figured in this and in my former paper, show conclusively, I think, that the early and late gastrulae derived from one of the first two blastomere of the *Cynthia* egg are approximately like the half of a normal gastrula and that neither in general form nor in the primordia of organs are they at all like entire gastrulae. Driesch says in his review of my work (1905, p. 659): »Ich habe bei meinem letzten Neapler Aufenthalt an $^1/_2$ Larven von *Phallusia* gerade wieder die Gastrulation als verkleinertes Abbild der Ganzgastrulabildung verlaufen sehen.« However he gives no detailed description of such $^1/_2$ gastrulae, and this is certainly necessary in order to establish his position. On casual observation the $^1/_2$ gastrulae of *Cynthia* frequently appear to be entire but with the axes unlike those of the normal gastrula; thus if one took no account of the organ primordia shown in Figs. 2 and 3, and supposed that the plane of bilateral symmetry lay at right angles to the first cleavage plane, these gastrulae might be mistaken for entire ones; especially in later gastrulae it frequently appears as if the typical endoderm invagination were taking place along that side next to the injured cell and that this is therefore the dorsal side and that the gastrula is consequently symmetrical and typical; but in not a single instance which I have examined would such a conclusion

Fig. 9.

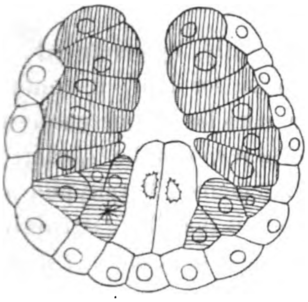


Fig. 10.

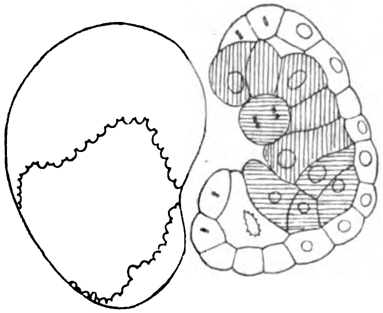


Fig. 11.

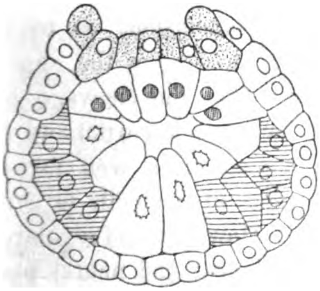


Fig. 12.

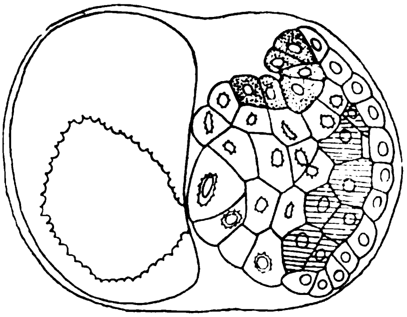


Fig. 9—12. Transverse sections of whole and of half gastrulae from Exp. 14 (same as Figs. 5—8). Figs. 9 and 11, whole gastrulae from uninjured eggs; Figs. 10 and 12 corresponding sections of half gastrulae from one of first two blastomeres.

Fig. 9. Section through open blastopore in posterior part of gastrula; blastopore and gastrocoel are bounded laterally by muscle cells, and ventrally by two caudal endoderm cells, on each side of which are small mesenchyme cells.

Fig. 10. Section of half gastrula in same plane as preceding; muscle mesenchyme and caudal endoderm cells of one side only are present; ectoderm cells have turned in so as to cover the median side of caudal endoderm cell.

Fig. 11. Section through neural groove in front of blastopore; the gastrocoel is bounded dorsally by six chorda cells, and ventrally by endoderm cells, two of which are continuous with the rows of caudal endoderm cells (see Fig. 9); on each side of these cells are the areas of mesenchyme.

Fig. 12. Section of half gastrula in same plane as preceding, showing the half-neural groove and one mesenchyme area, but no gastrocoel and no certain distinction between endoderm and chorda cells.

be justified. A study of the organ primordia shows that in every case the gastrula is unilateral and not bilateral, and that, with the exception of the ingrowth of a few ectoderm cells along the injured side and a few slight changes in the positions of some of the endoderm cells, the gastrulae derived from one of the first two blastomeres are like the half of a normal gastrula.

The typical ascidian gastrula is bilaterally symmetrical, its anterior half is visibly different from its posterior half, it contains recognizable primordia of neural plate, chorda, muscles and mesenchyme, and when Driesch is able to prove that in these respects the $^1/_2$ gastrula of *Phallusia* »kommt doch eben auf den allgemeinen Formtypus an« — then, and not before, it will be time to admit that the gastrulation of $^1/_2$ embryos of *Phallusia* is wholly unlike that of *Ascidia*, *Cynthia* and *Molgula*.

3. Larva.

In my former paper I summarized my conclusions as to the character of the larvae derived from $^1/_2$ or from $^3/_4$ of the egg as follows (p. 174): »In conclusion then I find that the cleavage and gastrulation of these half or three-quarter embryos is partial and the resulting larva incomplete, although the notochord is well formed and there is a tendency on the part of some of the cells to grow over and close up the open side of the larva. However this regulation never leads to the formation of a complete larva; the neural plate may close but it forms an abnormal sense vesicle; at the end of the tail the muscle cells extend over toward the injured side, but they do not form three rows of cells on each side of the notochord as in the normal larva; the mesenchyme likewise does not develop along the injured side and it is probable that only one atrial invagination is formed.« Again in explaining the closing of the embryo along the injured side I said (p. 169): »In later stages the muscle cells slowly extend over to the side of the tail on which they were originally lacking; this takes place especially at the hinder end of the tail, the overgrowth taking place around the end of the notochord and over its ventral side. In this way the right or left half embryo or larva tends to become complete, but I have never seen a case in which three rows of muscle cells were found on both sides of the notochord. Indeed I am not at all sure that this extension of the muscle cells around the end of the notochord is accompanied by any increase whatever in the number of cells or in the number of rows of cells . . . I have found it still more difficult to decide whether the trunk mesenchyme ever extends over to the side on which it was originally lacking, but I believe that this takes place only to a limited extent, if at all, and that CHABRY was right when he affirmed that only one atrial invagination is formed in these right or left half embryos.« It will thus be seen that I could find no evidence that these $^1/_2$ larvae ever became complete, though in the case of the tail there was a tendency for the muscle cells to extend around the end of the notochord to the injured side, apparently without any change in the number of muscle cells or in the number of rows of such cells. Again in my final summary (p. 218) I said: »A lateral half embryo is usually closed along the injured side; it has a head and a tail [not however complete]; a typical notochord, which is formed only from the chorda cells of the surviving side, and which is therefore composed of half the normal number of cells; an atypical neural plate and sense vesicle, formed only from the typical neural plate cells of the surviving side; one typical mesenchyme area in which the atrial invagination of one side is formed, and three typical rows of muscle cells along on side of the notochord, but none along the injured side. In the latest stages to which these lateral embryos were reared (corresponding to the period of metamorphosis in normal larvae) the muscle cells have begun to grow around the hinder end of the notochord to the side on which they were lacking but in no case are the three rows of the normal embryo present on this side. Probably only one atrial invagination and one papilla are ever formed in these lateral embryos. These are therefore half embryos in which some cells have grown over from the uninjured to the injured side, but in which absolutely no change has taken place in the potency of the individual cells or of the different ooplasmic substances[1]).«

---

[1]) DRIESCH has made this last sentence the text of a severe arraignment of me; he admits that there has been no change in the potency of the ooplasmic substances, but treats with contempt the statement that there is no change in the potency of the individual cells. I am not concerned to prove myself omniscient and infalliable, and if I could detect any error in this statement I would gladly correct it. Since however it is strictly true that the only modification of these cells is one of position, and since they never give rise to the organs of the missing side as I proved in my former paper and as I shall show in detail later, I see no cause for modifying it, unless indeed »potency«, in the terminology of DRIESCH, refers only to position, and not to the possibilities of development as regards organs and tissues.

I have quoted thus at length the conclusions of my former paper as to the structure of the $^{1}/_{2}$ larvae because Driesch has subjected them to a scathing, and, as I believe, wholly unjustifiable criticism. Referring to my statement that in the later stages of development muscle cells grow around the hinder end of the notochord, Driesch says (p. 659): »Dieser Prozeß geht freilich nicht zu Ende . . . Was heißt aber dieses? Es heißt, daß die eigentliche aus einer $^{1}/_{2}$ Blastomere gezogene Larve auch nach Conklin sogar in Hinsicht auf ihre Muskeln nahezu ganz war. Nun erfahren wir ferner noch, daß Neuralrohr, Chorda und Sinnesblase, ja Kopf und Schwanz überhaupt, ebenfalls ganz waren: Was wollen wir also weiter? Aber alles war doch halb und typisch Mosaik, sagt uns Conklin.«

As a matter of fact I never said no thought that the $^{1}/_{2}$ larva of the ascidian was nearly complete as regards its muscles, nor that head and tail generally were in any case entire, and I challenge Driesch to show that I have said this, or that it is true. On the contrary I maintained that all the organs of the right side are missing in a larva derived from a left blastomere, and that in a right half-larva all the organs of the left side are lacking, while organs which normally lie in the mid-line are present in both right and left half-larvae, in more or less modified form.

I now find that I guarded rather too carefully my oft-repeated assertion that the $^{1}/_{2}$ blastomere gives rise only to a half-larva, and that I was too much impressed with the possible regulation which might result from the overgrowth of cells from the uninjured to the injured side, if the development were not so rapid; for although the $^{1}/_{2}$ larvae always lacked the organs of the missing side I granted the possiblility (p. 207) that if the development were not so rapid the larva might become complete; but it is evident throughout the whole paper that I placed no emphasis upon this possibility and much upon the fact that the $^{1}/_{2}$ larvae are in all cases incomplete.

Since I have apparently failed to make these facts perfectly plain and sufficiently explicit, and since I have not hitherto published any figures of actual sections of these $^{1}/_{2}$ larvae, I submit herewith a number of camera drawings of such sections. I have carefully studied sections of hundreds of these partial larvae in all stages of development and I have never seen an instance in which the organs of one side (muscles, mesenchyme, atrial invagination) were present in a larva derived from the blastomere of the

Fig. 13.

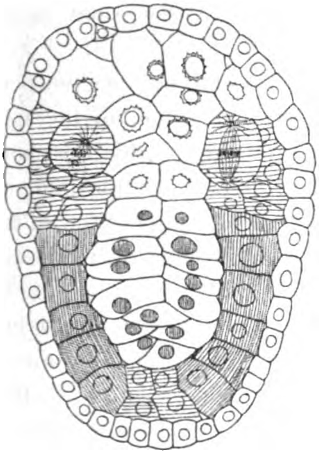


Fig. 14.

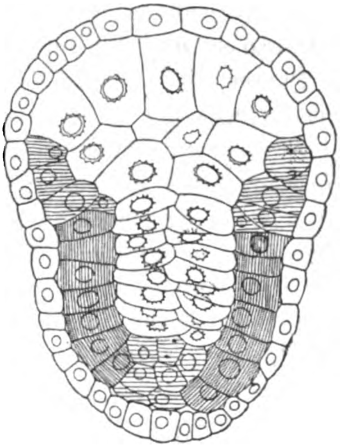


Fig. 15.

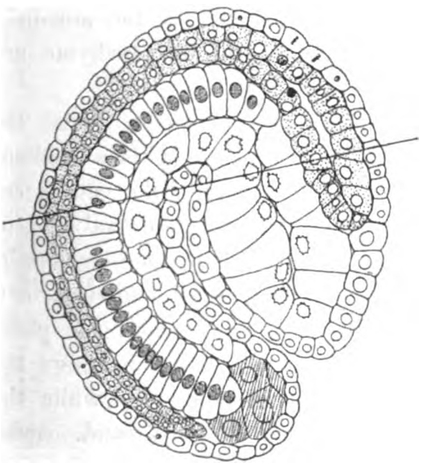
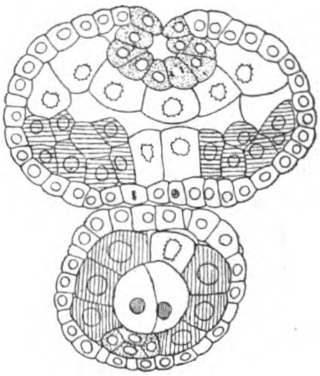


Fig. 16.



Figs. 13 and 14. Frontal-longitudinal sections of slightly elongated larvae from uninjured eggs of Exp. 14 (Laid 5:15; spurted 6:15; fixed 9:45 P.M.).

Fig. 13. Section at level of notochord, which consists of two rows of cells; at posterior end of notochord are a few caudal mesenchyme cells; on each side a row of muscle cells and in front of these an area of mesenchyme on each side.

Fig. 14. Section in same plane as preceding but nearer the ventral side and passing through the caudal endoderm, which also forms two rows of cells; muscle and mesenchyme cells essentially the same as in the preceding figure.

Figs. 15 and 16. Sections of entire larvae with elongated tail, from uninjured eggs of Exp. 16 (Laid 5; spurted 6; fixed 11 P.M.).

Fig. 15. Median-longitudinal section showing notochord as a single row of cells; on the ventral side of this is a row of caudal endoderm cells; on the dorsal side, the nerve cord. At the posterior end of the notochord is a group of mesenchyme (or muscle) cells; anteriorly the nerve tube forms a sense vesicle in which one sense spot is shown.

Fig. 16. Cross section in plane of straight line in Fig. 15, showing the closing of the sense vesicle, and an area of mesenchyme on each side of the gastrocoel; in the tail there are three rows of muscle cells on each side of the notochord, while the nerve cord marks the dorsal mid-line and the caudal endoderm the ventral mid-line.

opposite side. The study of these sections confirms and extends the results which I had reached in my former paper, and in no single respect does it contradict them.

Figures 13 and 14 represent frontal-longitudinal sections of normal larvae during the early stages of the elongation of the tail; Fig. 13 is a section at the level of the notochord which is here composed of two rows of cells, one on each side of the mid-line; on each side of the notochord are six large muscle cells, in front of the muscle cells on each side is mesenchyme of the trunk, in which the atrial invaginations later appear, and between the muscle cells at the posterior end of the notochord is a small group of caudal mesenchyme cells. These muscle and mesenchyme cells which contain an orange pigment in the living animal stain much more deeply than any other cells of the larva, so that they are always clearly distinguishable from the other cells. Figure 14 is a section nearer the ventral surface than the preceding one, showing the caudal endoderm extending into the tail as a double row of cells, one on each side of the mid-line; the muscle cells, trunk mesenchyme and caudal mesenchyme are essentially the same as in the preceding figure.

Figures 15 and 16 are sections of normal larvae in which the tail has grown out to a considerable length; Fig. 15 is a median-longitudinal section showing the notochord between the caudal endoderm on the ventral side and the nerve tube on the dorsal side, at the hinder end of the tail is a group of caudal mesenchyme (or muscle) cells, while in the trunk region is found the gastral endoderm and sense vesicle; Fig. 16 is a transverse section in about the plane indicated by the straight line in Fig. 15; in the trunk it shows the sense vesicle, gastral endoderm and trunk mesenchyme, while the section of the tail shows the notochord, nerve cord and caudal endoderm in the median plane and three muscle cells on each side; the double rows of chorda cells and of caudal endoderm cells of the preceding stage have here interdigitated more or less completely, thus forming a single row of cells in each structure, while the nerve cord is composed of four cells in cross section. Similar cross sections of the tail of a slightly older larva, and also of a free-swimming one, are shown in Figs. 24 and 25.

Figures 17 and 18 are frontal-longitudinal sections of partial larvae from the same experiment as Figs. 13 and 14; Figs. 19 and 20 are similar sections of slightly older larvae. Figure 17 shows the muscle and mesenchyme cells on one side only; there is only one

Fig. 17.

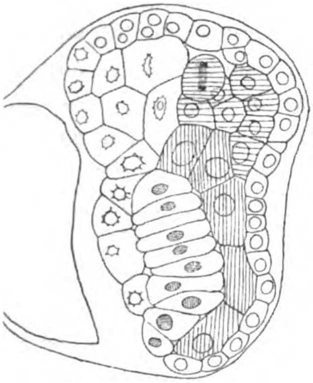


Fig. 18.

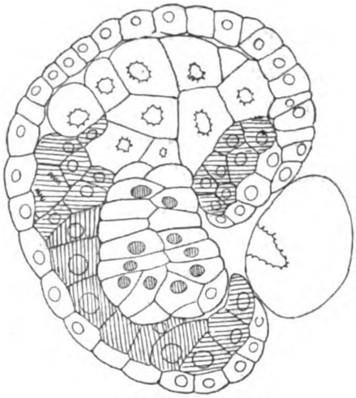


Fig. 19.

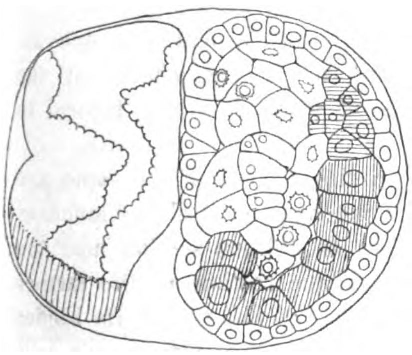


Fig. 20.

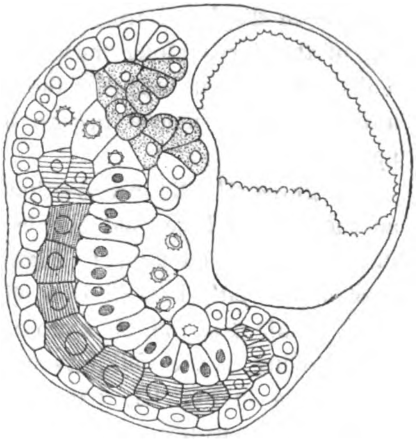


Fig. 17. Frontal-longitudinal section of half larva from Exp. 14 (same as Figs. 13 and 14); chorda composed of one row of cells instead of two (see Figs. 13 and 18); on the right side of the chorda is a row of muscle cells, on the left, a row of caudal endoderm; one mesenchyme area lies anterior to the muscle row.

Fig. 18. Frontal-longitudinal section of $^3/_4$ larva from Exp. 14, the right posterior blastomere having been killed in the 4-cell stage. The larva is like a normal one (Fig. 13), save that the muscles and ectoderm which normally would have come from the dead blastomere are lacking.

Fig. 19. Frontal-longitudinal (oblique) section of a half larva from Exp. 15 (Laid 5:15; spurted 6:15; fixed 10:30 P.M.); one mesenchyme area; muscle cells chiefly on right side, though one or two have turned in on injured side; ectoderm has nearly inclosed the larva; chorda and endoderm cells not certainly distinguishable.

Fig. 20. Frontal-longitudinal section of half larva from Exp. 15; muscle cells on left side of notochord, ventral endoderm, on right; ectoderm at anterior and posterior ends turning in over injured side; neural groove well formed at anterior end; one mesenchyme area in trunk.

row of chorda cells and on the left side of these is a row of caudal endoderm cells which has been pushed into this position by the general closing of the injured side. Figure 18 is a left-anterior $^3/_4$ larva, the right-posterior blastomere having been killed in the 4-cell stage; the muscle cells are present only on the left side of the chorda, the trunk mesenchyme is present, however, on both sides and the chorda is composed of two rows ot cells as in the normal larva; this agrees entirely with my former account that the chorda and trunk mesenchyme are derived entirely from the anterior quadrants, the muscles from the posterior quadrants. In Fig. 19 the muscle and mesenchyme cells are confined almost entirely to the right side though one or two of these cell are shown along the injured side at the posterior end; in this section the ectoderm cells have nearly covered the injured side, but the larva is in no other regard even approximately complete. Figure 20 is a frontal-longitudinal section of a slightly more advanced larva, showing a single row of chorda cells, on the right of which is a row of caudal endoderm cells which have been displaced from their ventral position by the general closing of the larva along the injured side; muscle and mesenchyme cells are present only on the left side of the notochord; at the anterior end a neural groove is present which is nearly typical in appearance.

Figures 21—23 are sections of a single larva of the same age as Figs. 15 and 16, but the larva is not bent on itself as much as in the last named figures. Figure 21 is an oblique section near the base of the tail; Fig. 22 is a slightly oblique section a little farther back, while Fig. 23 is a cross section of the tail nearer its hinder end. Owing to the obliquity of the section, parts of four muscle cells are shown in Fig. 21, but in Figs. 22 and 23 there are but three muscle cells, exactly as in the right half of a normal larva. The neural tube (or rather neural plate), notochord and caudal endoderm cells are present in half their typical number and they lie not in the mid-line as in the normal larva, but along the injured side. Owing to the considerable number of cells in the trunk it is not always possible to identify all of the cells seen in a cross section of this region, but in the tail where the number of cells is small it is possible to identify every cell and to show in the most conclusive manner that there is no restitution of the organs of the missing side. In the closing of the $^1/_2$ larva, the ventral mid-line which is marked by the caudal endoderm cells comes into contact with the dorsal mid-line which is marked by the neural plate cells, while the muscle cells lie entirely on one side of the notochord and not on both sides, as in the normal larva. These $^1/_2$ larvae are exactly such as would result if a fully formed larva were cut in two along the median plane and the cut edges of each half then came together, the dorsal and ventral mid-lines joining.

Fig. 21.

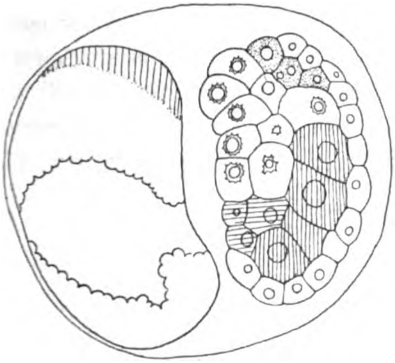


Fig. 22.

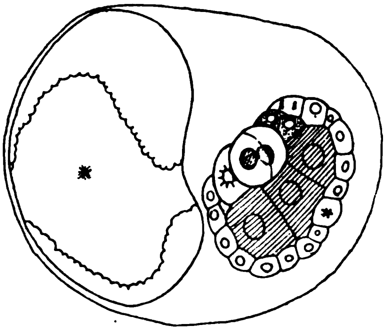


Fig. 23.

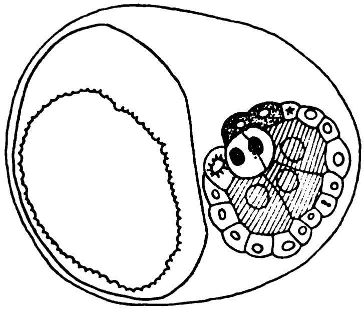


Fig. 24.

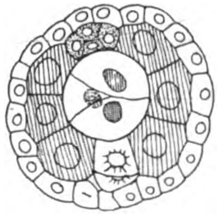
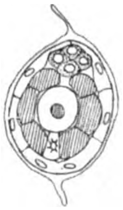


Fig. 25.



Figs. 21—23. Three sections through one half-larva from Exp. 16 (Laid 5: spurted 6; fixed 11 P.M.).

Fig. 21. Obliquely transverse section near base of tail, showing muscle cells on one side and three nerve cord cells on dorsal side; endoderm protrudes freely on injured side.

Fig. 22. Transverse section of tail five sections behind Fig. 21, showing nerve cord, notochord and caudal endoderm on injured side, and three muscle cells on the other; ectoderm has not inclosed injured side.

Fig. 23. Transverse section of tail four sections behind Fig. 22, showing nerve cord in contact with caudal endoderm cells on one side of notochord and three muscle cells on other side.

Fig. 24. Cross section of tail of entire larva from uninjured egg of Exp. 16.

Fig. 25. Cross section of tail of entire free-swimming larva, just before metamorphosis.

Figures 26 and 27 are cross sections of the trunk and tail of one and the same normal larva, 24 hours after fertilization; Fig. 27 being three sections posterior to Fig. 26. The tail is here wrapped round the body in a horizontal plane and in Fig. 26 it is cut twice, the extremity of the tail being shown in section on the right side of the body, and a more proximal portion being shown on the left. In both of these figures the dorsal and ventral mid-lines in the tail are marked by the neural plate and caudal endoderm, respectively, while three muscle cells are shown on each side of the notochord, as in all previous stages. In the trunk the sense vesicle, gastral endoderm and trunk mesenchyme are plainly visible. This is the oldest larva which I have studied; in fact at this age (24 hrs.) many normal larvae have already undergone metamorphosis. Figures 28—30 are three sections of a $^1/_2$ larva of about 14 hrs. In the trunk region this larva is so twisted that it is difficult to describe accurately the different axes, however, Fig. 28 lies nearest the ventral side, Fig. 30 nearest the dorsal. Throughout the whole series of sections the mesenchyme is found only on the right side and in Fig. 29 the heavy line leading through the ectoderm into the mesenchyme may possibly represent the atrial invagination. The anterior end of the nerve tube lies on the side of the larva next to the injured blastomere and from this position the nerve tube runs forward and toward the right side, dipping under the mesenchyme area shown in Fig. 30 and finally joining the neural plate in the tail, as shown in Fig. 30. In this larva the ectoderm has almost entirely inclosed the larva on the injured side, the endoderm coming to the surface only over a small area (Fig. 30). The nerve tube and endoderm are here abnormal in

Fig. 26.

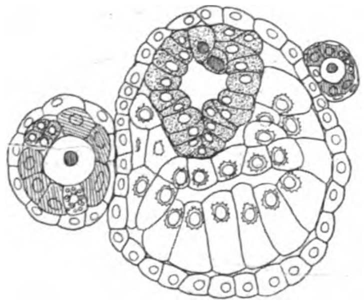
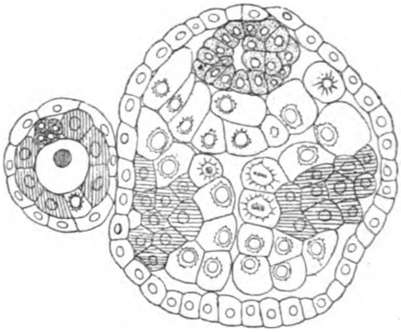


Fig. 27.



Figs. 26, 27. Cross sections of one entire larva from an uninjured egg of Exp. 33 (Laid 5:15; spurted 7; fixed 5 P.M. next day).

Fig. 26. Section through sense vesicle and spots, showing a section through the tip of the tail on the right, and through middle of tail on left.

Fig. 27. Third section posterior to Fig. 26, showing a mesenchyme area on each side; structure of tail as in preceding figures.

appearance; the mesenchyme is present only as a single area on the right side, and this shows at once that this larva is unilateral. This is shown in the clearest possible manner by the structure of the tail. Here, as in Fig. 22 and 23, the nerve cells and the caudal endoderm

Fig. 28.

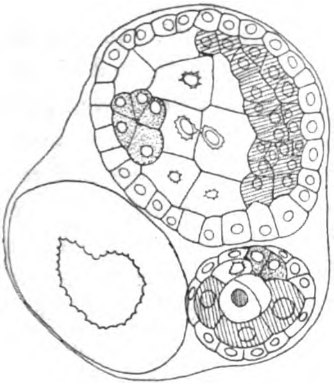


Fig. 29.

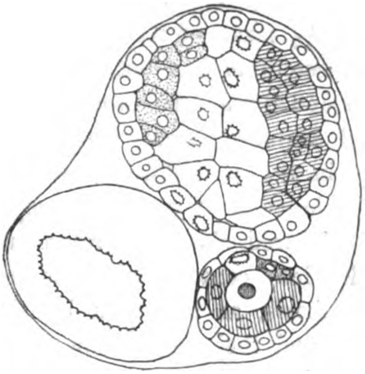


Fig. 30.

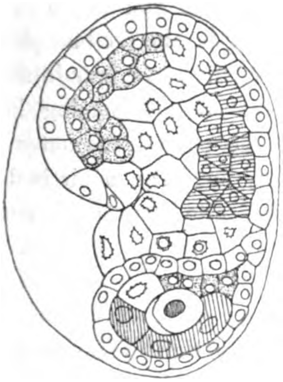
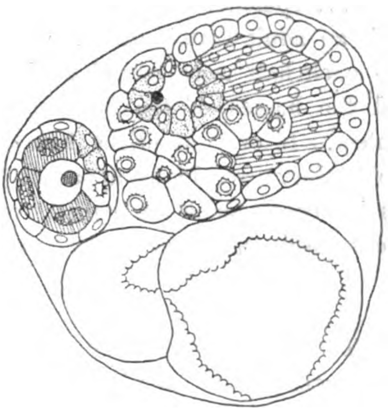


Fig. 31.



Figs. 28—30. Three sections of same half-larva from Exp. 17 (Laid 5:15; spurted 6:15; fixed 7 A. M.); larva almost entirely inclosed by ectoderm; larva twisted so that neural tube, beginning in Fig. 28 on left side, runs around anterior end in Figs. 29 and 30 and then dips under mesenchyme area of Fig. 30 to join neural cord in tail.

Fig. 28. In head, neural tube on left side, mesenchyme on right; in tail, nerve cord and caudal endoderm cells in contact on one side of notochord three muscle cells on the other side.

Fig. 29. Same as preceding; atrial invagination (?) into mesenchyme area.

Fig. 30. Section through junction of head and tail; nerve cord and mesenchyme as in preceding; endoderm not entirely covered by ectoderm.

Fig. 31. Cross section of half-larva from Exp. 33 (Laid 5:15; spurted 7; fixed 5 P. M. next day); showing well formed sense vesicle and spot; endoderm uncovered by ectoderm; one mesenchyme area in tail; neural cord and caudal endoderm cells in contact on one side of notochord; three muscle cells on other side; muscle cells are becoming vacuolated.

cells have come into contact along the injured side, thus bringing the dorsal and ventral mid-lines together, while the three rows of muscle cells lie on the other (uninjured) side.

Finally Fig. 31 represents a cross section through head and tail of a $^1/_2$ larva of the same stage as Figs. 26 and 27. In this larva the ectoderm has not inclosed the body along the injured side, but the endoderm cells protrude freely; the sense vesicle is well formed and contains a sense spot, but only one mesenchyme area is present. As in the preceding figures, the section of the tail shows that the muscle cells of one side only are present, and that the closure of the injured side has brought the neural plate and caudal endoderm cells into contact. This larva is already entering upon its metamorphosis, as is shown by the vacuolated condition of the muscle cells and their nuclei and yet, up to this last stage of the larva life, there is no trace of the restitution of the organs of the missing side.

These sections show that, so far as the end result is concerned, the development of a $^1/_2$ blastomere of the ascidian egg is the same as if an adult vertebrate were to be cut in two in the median plane and each half were to roll up into a cylinder, the cut edges joining and the halves of the sternum and vertebral column coming into contact. If only ascidians had paired legs the partial character of the $^1/_2$ larva would be apparent to every-one, but even as it is this partial character is sufficiently evident. »Aber alles war doch ganz und typisch, sagt uns Driesch.«

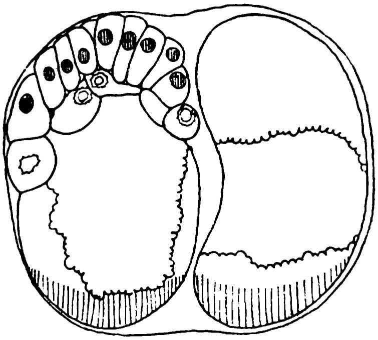
The opinion expressed in my former paper that restoration of the organs of the missing side might possibly take place if the development were not so rapid, does not appear to be justified; for since the dorsal mid-line is marked by differentiated cells which normally give rise to half of the nerve tube, while the ventral mid-line is likewise marked by a row of caudal endoderm cells, the closure of the larva along the injured side can only serve to bring these already diffenrentiated cells into contact. Therefore the only possible way in which muscle cells could grow from the uninjured side would be around the hinder end of the notochord; such a growth does take place, but to so limited an extent as to be of no significance in the restitution of the muscles of the missing side. In fact this very slight movement of the muscle cells around the end of the notochord is only a part of the general turning in of cells along the injured side, which as DRIESCH has said (1895), may be only a phenomenon of surface tension, and which in this case does not lead to the restitution of any of the organs of the missing side.

The differentiations of the different types of cells (chorda, endoderm, muscle, mesenchyme and neutral plate) is apparently so great that they can give rise to no other types of structures than those which they form under normal conditions, and the same may be said of the different ooplasmic substances (ectoplasm, endoplasm, chordaneuroplasm, myoplasm, chymoplasm) of the earliest cleavage stages; this is, after all, the chief result of my work.

4. Quarter Larvae.

Regarding the potency of the anterior or posterior $^2/_4$ blastomeres, or of any $^1/_4$ blastomere, the evidence seems to me complete that in *Cynthia* und *Molgula* they never give rise to an entire larva, but rather that they produce only those organs or parts of organs which would have come from them if they had remained a part of the entire egg. I treated this matter at length in my former paper, publishing thirty detailed figures of such partial larvae, and it does not seem necessary to add to the evidence there presented until that evidence may be impugned. DRIESCH, it is true, maintains that the »typisch ganz entwickelte kleine Larve«, represented in his Fig. 16, was derived from a $^1/_4$ blastomere, though he does not know from which quadrant it came. The figure of this larva shows what is apparently notochord and endoderm, surrounded by a layer of ectoderm, but no other structures. I have shown, in *Cynthia* and *Molgula*, that endoderm, chorda and neutral plate cells may develop from an anterior quadrant, but that a tail, with its muscles and caudal endoderm is never present, that a typical neural tube never forms and that the larva is never bilaterally symmetrical. In view of the abundance of detailed evidence which I presented upon this point, I ventured to express a doubt as to whether the solitary $^1/_4$ larva obtained by DRIESCH was really derived from a $^1/_4$ blastomere. This doubt DRIESCH has happily answered, but he has furnished no evidence that his $^1/_4$ larva is really a »typisch ganz entwickelte kleine Larve«; that it is bilaterally symmetrical, and that it possesses nerve tube, muscles, mesenchyme and caudal endoderm. The mere presence of a notochord is not sufficient to establish the conclusion that the $^1/_4$ larva is complete; I have shown, in my former papers, that the notochord of the ascidian larva always arises from the anterior quadrants and that a more or less typical notochord may be formed from one of these quadrants. In fact the chorda cells may survive in a $^1/_4$ larva when all the other cells have perished; Fig. 32 shows such a case; this egg was injured during the second cleavage in such a way as to leave only a portion of the anterior quadrant of the left side capable of further development; a few nuclear divisions have occurred in the ventral (ectodermal) half of this quadrant, but the only cells with cell walls are those shown in the figure. These cells, both by their histological character and by their location, may be clearly distinguished as chorda and endoderm cells; furthermore, these chorda cells are arranged in a linear series, and if the structure thus formed may be held to constitute a notochord, then this is the only organ of this surviving quadrant. Certainly the presence of a notochord is not sufficient evidence that a larva is complete; it does not establish the presence of neural tube, mesenchyme nor muscles and it does not prove that the larva is bilaterally symmetrical.

Fig. 32.



Horizontal section through partial embryo from Exp. 16 (Laid 5; spurted 6; fixed 11 P. M.), in which the egg was injured during the second cleavage so that only a portion of the left anterior quadrant developed, giving rise only to chorda and endoderm cells.

Driesch has furnished no evidence that his $^1/_4$ larva was really a complete one, and while we may grant his claim that he is able to distinguish a quarter of an egg from a half, there is good reason to conclude that he has not distinguished a quarter, or a half larva, from a whole one.

Of course it may be true, as Driesch suggests, that in respect to the presence of different ooplasmic substances¹) and the potency of $^1/_2$ and $^1/_4$ blastomeres *Phallusia* differs totally from *Ascidia*,

---

¹) Driesch says (p. 661): »Das Ei der *Phallusia* ist glashell und läßt nichts von den verschiedenen Stoffen Conklin's erkennen.« This does not prove that these substances are not present in *Phallusia*; the mesoplasm, which is orange colored in *Cynthia*, is colorless and transparent in *Ciona* and *Molgula*, but it may be clearly differentiated from the remainder of the egg by its greater affinity for plasma stains, as well as by its greater transparency in life.

Cynthia and *Molgula*; but considering the well known resemblances between these genera in cleavage, gastrula and larva, this seems most improbable, and at least demands more evidence in its support than has yet been offered.

Finally, DRIESCH says (p. 664): »Selbst wenn CONKLIN's Arbeiten durchaus einzig zu Recht bestehen und ein Teil der meinigen positiv falsch ist, wäre also die Ascidienentwicklung ein ‚Mosaik' nur soweit sie von typischen verteilten Eibau-Stofflichkeiten abhängig ist; in Hinsicht ihrer Zusammensetzung aus zwei Hälften wäre sie aber kein Mosaik, sondern in dieser Hinsicht wäre auch beim Ascidienkeim die prospective Bedeutung der Elemente jedes Elementarorgans eine Funktion ihrer Lage.«

I am pleased to find that DRIESCH adopts, although only in part and conditionally, the view with which I summed up my former paper (p. 221); viz. »The development of ascidians is a mosaic work because there are definitely localized organ-forming substances in the egg; in fact the mosaic is one of organ-forming substances rather than of cleavage cells.« It is interesting to observe, also, that the dictum, which was first applied to cases in which the cleavage was a »mere sundering of homogeneous material capable of any fate«, is now also applicable to eggs in which the different blastomeres and ooplasmic substances are so highly specialized that each is capable of one particular fate only.

In conclusion, a few brief references to minor criticisms which have been brought against me: Assuming, as DRIESCH does, that the $^1/_2$ larvae of *Cynthia* are entire, he finds that I am guilty of a Petitio principii, in that I treat the »prospektive Bedeutung« of certain cells as a »prospektive Potenz«. But if these $^1/_2$ larvae are really partial, is it not evident that it is DRIESCH who begs the question, as well as the evidence, in his assumption that they are complete?

DRIESCH finds that I am ignorant of the literature¹), that I do not distinguish between defects due to lack of typical constituents

---

¹) He complains that I have not cited two of his papers which he thinks important as bearing on this subject: »Er berücksichtigt dabei aber meine ‚Analytische Theorie' (1894) und ‚Organisation des Eies' (1896), in welcher alle neueren Ergebnisse der Blastomerenforschung vorausgesagt sind, mit keinem Worte.« One of these papers (Organisation des Eies) was referred to in two previous papers in my series on the ascidian egg (1905¹, 1905²), both of which DRIESCH lists in his bibliography. Must I continue to quote this paper as a sort of pious preamble to everything I write?

and those due to lack of regulation, (a subject to which much of my former paper is devoted), and that, by sharply contrasting the determinism or indeterminism of cleavage cells with the determinism of the general development, I have fundamentally confused the two. I feel that these criticisms are unjust, but whether they are or not, I hope that they may not be allowed to obscure the main question, viz. whether the individual blastomeres of the ascidian egg, up to the 4-cell stage, may give rise to entire larvae, or not. Driesch is convinced that they do; I have presented evidence that they do not, and the only question of importance in this conflict of opinion is as to the relative weight and value of the evidence offered by each of us.

Summary.

1) A reexamination of stained and mounted preparations of the eggs of *Cynthia* (*Styela*) partita and of *Molgula* manhattensis shows that the cleavage of a surviving $^1/_2$ blastomere is the same as if that blastomere were still part of the entire egg, save for slight changes in the direction of division and in the resulting positions of some of the cells. The rhythm of cleavage; the size, histological character and lineage of every cell; the isolation of particular ooplasmic substances in particular cells, are all the same as in the corresponding half of a normal egg. Such cleavage forms are never bilaterally symmetrical, and, if the surviving blastomere is uninjured, they are no more »regellos-solid« than in the normal egg. These results confirm those previously reached by Chabry, Crampton and myself.

2) Serial sections, as well as entire preparations, of the early and the late $^1/_2$ gastrulae of *Cynthia* show that each is approximately like the half of a normal gastrula, and that neither in general form nor in the location of organ primordia are they at all like entire gastrulae. The primordia of muscles and mesenchyme are found on one side only and in no case are these $^1/_2$ gastrulae bilateral. Except for the ingrowth of ectoderm cells along the injured side and slight changes in the position of some of the endoderm cells, each of these $^1/_2$ gastrulae resembles the corresponding half of a normal gastrula. These results confirm and extend those previously obtained by Chabry and myself.

3) Serial sections of $^1/_2$ larvae show that up to the time of the metamorphosis all the organs of the right side are missing in a larva

derived from a left blastomere, and that all the organs of the left side are lacking in a right half-larva, while organs which normally lie in the mid-line are present in both right and left half-larvae in more or less modified form. In the head region the ectoderm usually overgrows the injured side more or less completely and a sense vesicle may be formed from the neural plate cells, but the mesenchyme (and consequently the atrial invagination) of the injured side is entirely lacking. In the tail the dorsal mid-line (marked by neural cord cells) comes into contact with the ventral mid-line (marked by caudal endoderm cells), while the three typical rows of muscle cells lie on one side only of the notochord, and not on both sides as in a normal larva. These $^1/_2$ larvae are such as would result if a fully formed larva were cut in two in the median plane and the cut edges of each half then came together, the dorsal and ventral mid-lines joining. These results confirm and extend those previously reached by CHABRY and myself, but they show that the opinion expressed in my former paper that the organs of the missing side might possibly be restored if the development were not so rapid, was not justified. The specialization of the different types of cells (ectoderm, endoderm, muscle, mesenchyme, chorda, neural plate) and of the different ooplasmic substances (ectoplasm, endoplasm, myoplasm, chymoplasm, chorda-neuroplasm) is apparently so great that they can give rise to no other types of structures than those which they form under normal conditions; and, conversely, embryos which lack any of these cells or substances, lack also the organs or parts of organs which would normally come from them. These visibly different ooplasmic substances are therefore »organ-forming substances« and the areas in which they are located are »organ-forming germ regions«.

University of Pennsylvania, March 28, 1906.

Zusammenfassung.

1) Eine Durchmusterung gefärbter Dauerpräparate der Eier von *Cynthia (Styela) partita* und von *Molgula manhattensis* zeigt, daß die Teilung eines überlebenden $^1/_2$-Blastomers ebenso verläuft als ob das Blastomer noch immer Teil des ganzen Eies wäre, abgesehen von leichten Änderungen der Teilungsrichtung und der resultierenden Stellungen einiger Zellen. Der Teilungsrhythmus, die Größe, der histologische Charakter und die Abstammung der einzelnen Zellen, die Isolation spezieller Substanzen des Eiplasmas in besonderen Zellen, alles ist ganz ebenso wie in den korrespondierenden Hälften eines normalen Eies. Solche Teilungsformen sind niemals bilateral symmetrisch, und, wenn die überlebende

Teilhälfte nicht verletzt wurde, sind sie ebensowenig »regellos solid« als im normalen Ei. Diese Ergebnisse bestätigen die früher von Chabry, Crampton und mir selbst erhaltenen.

2) Serienschnitte sowohl wie Totalpräparate junger und alter Gastrulae von *Cynthia* zeigen, daß jede annähernd der Hälfte einer normalen Gastrula entspricht, und daß sie weder in der allgemeinen Form noch in der Unterbringung der Primordialorgane irgendwie den Ganzgastrulae ähneln. Die ersten Anlagen von Muskulatur und Mesenchym finden sich lediglich auf einer Seite und in keinem Falle sind diese Halbgastrulae zweiseitig. Abgesehen von dem Einwachsen der Ectodermzellen entlang der Verletzungsseite und leichten Stellungsänderungen der Ectodermzellen ähnelt jede dieser Halbgastrulae der entsprechenden Hälfte einer normalen. Diese Ergebnisse bestätigen und erweitern die schon vorher von Chabry und mir selbst erhaltenen.

3) Serienschnitte von Halblarven zeigen, daß bis zur Zeit der Metamorphose alle rechtsseitigen Organe in einer Larve aus einer linken Hälfte und alle linksseitigen in einer rechten Halblarve fehlen, während normalerweise in der Mittellinie gelegene Organe in beiden, rechten und linken, Halblarven in mehr oder weniger modifizierter Form vorhanden sind. In der Kopfregion überwächst das Ectoderm die verletzte Seite mehr oder weniger vollständig und ein Sinnesbläschen kann von den Zellen der Nervenplatten gebildet werden, aber das Mesenchym (und infolgedessen auch die Atrium-Einstülpung) der verletzten Seite fehlt vollständig. Am Schwanz kommt die dorsale Mittellinie (kenntlich durch die Nervenstrangzellen) zur Berührung mit der ventralen Mittellinie (kenntlich durch die Zellen des Schwanz-Entoderms), während die drei typischen Reihen von Muskelzellen nur auf einer Seite des Notochords liegen und nicht auf beiden, wie in einer normalen Larve. Diese Halblarven sind so beschaffen, wie das Ergebnis einer in der Medianebene erfolgenden Zerschneidung einer ausgebildeten Ganzlarve und einer nachherigen Vereinigung der Schnittränder auf jeder Hälfte derart, daß sich dorsale und ventrale mittellinige vereinigen. Diese Ergebnisse bestätigen und erweitern die vorher von Chabry und mir erhaltenen, aber sie zeigen, daß die in meiner früheren Arbeit von mir geäußerte Ansicht, die Organe der fehlenden Seite könnten möglicherweise wieder hergestellt werden, wenn die Entwicklung nicht eine so rapide wäre, der Berechtigung entbehrt. Die Spezialisation der unterschiedlichen Zellentypen (Ectoderm, Entoderm, Muskel-, Mesenchymanlage, Chorda, Nervenplatte) und der verschiedenen Substanzen des Eiplasmas (Ectoplasma, Endoplasma, Myoplasma, Chymoplasma, Chorda-Neuroplasma) ist anscheinend so groß, daß sie keinen andern Strukturtypen zum Ursprung dienen kann, als den unter normalen Bedingungen gebildeten, und dementsprechend fehlen auch Embryonen, denen gewisse von diesen Zellen oder Substanzen abgehen, auch die Organe oder Organteile, welche normalerweise von jenen abstammen. Diese sichtlich verschiedenen Eiplasmasubstanzen sind demnach »organbildende Substanzen« und die Bezirke, in welchen sie untergebracht sind, »organbildende Keimbezirke«.

References.

Barfurth, D. (1893), Halbbildung oder Ganzbildung von halber Größe? Anat. Anz. VII.

Chabry, L. (1887), Contribution à l'embryologie normal et teratologique des Ascidies simples. Journ. Anat. et Physiol. XXIII.

Conklin, E. G. (1905[1]), The Organization and Cell-Lineage of the Ascidian Egg. Journ. Acad. Nat. Sci. Philadelphia. XIII.

—— (1905[2]), Organ-Forming Substances in the Eggs of Ascidians. Biol. Bull. VIII.

—— (1905[3]), Mosaic Development in Ascidian Eggs. Journ. Exp. Zool. II.

Crampton, H. E. (1897), The Ascidian Half-Embryo. Ann. New York Acad. Sci. X.

Driesch, H. (1892), Entwicklungsmechanische Studien. I. Zeitschr. f. wiss. Zool. LIII.

—— (1895), Von der Entwicklung einzelner Ascidienblastomeren. Arch. Entw.-Mech. I.

—— (1903), Über Änderung der Regulationsfähigkeiten im Verlauf der Entwicklung bei Ascidien. Arch. Entw.-Mech. XVII.

—— (1905), Die Entwicklungsphysiologie von 1902 bis 1905. Ergebnisse der Anat. und Entwicklungsgeschichte. XIV.

Hertwig, O. (1892), Urmund und Spina bifida. Arch. mikr. Anat. XXXIX.

Roux, W. (1892), Über das entwicklungsmechanische Vermögen jeder der beiden ersten Furchungszellen des Eies. Gesammelte Abhandlungen. II. 1895.

Weismann, A. (1892), Das Keimplasma. Jena.

THE EMBRYOLOGY OF FULGUR: A STUDY OF THE INFLUENCE OF YOLK ON DEVELOPMENT.

BY EDWIN G. CONKLIN,
PROFESSOR OF ZOOLOGY, UNIVERSITY OF PENNSYLVANIA.

I. INTRODUCTION.

1. GENERAL.

It is generally believed that the accumulation of yolk in the eggs of certain animals has greatly modified their original manner of development. For example, selachians, reptiles and birds are probably derived from animals in which the eggs contained much less yolk than at present in these classes, and in which the development was different in many respects from that which now obtains among these forms. The effect of the loss of yolk upon development has been studied experimentally by Morgan (1893), but the influence of an increase in the amount of yolk, while the protoplasmic portion of the egg remains the same, has not been studied experimentally, and may perhaps lie beyond the test of experiment.[1] Such experiments, however, nature has performed in several different phyla of animals, but nowhere are natural conditions more favorable for a study of the influence of yolk on development than among the mollusks. Brooks (1879) long ago called attention to this fact, though it has not been reinvestigated in the light of the modern cellular study of development.

The comparison of the development of large eggs with that of small ones, with especial reference to the organization of the egg and the history of the cleavage cells, should be of considerable general interest. Does a great accumulation of yolk change the localization of morphogenetic processes and substances in the egg? Does it alter the developmental history and destiny of the blastomeres? Is the yolk itself localized in any definite germinal region of the egg? In what manner does a great mass of yolk alter the gastrulation and later embryonic development? These are questions upon which a detailed comparison of the normal development of large and of small eggs might be expected to shed some light. Undoubtedly experiments, if they could be successfully performed, would be of great value in answering these questions, but in the case of the gasteropods which I have studied I have not found it possible to test these questions experimentally.

---

[1] The fusion of different eggs to form giants, such as occurs abnormally in certain cases, is not a case in point, since the relative amount of yolk and protoplasm remains the same in the fused eggs as in the single ones.

The development of gasteropods is extraordinarily varied in respect to the manner of nourishing the embryo and larva. In some cases, and these presumably the more primitive, the eggs are small and contain little yolk and the free swimming larvæ (veligers) are set free at an early age to shift for themselves (*e.g.*, *Crepidula plana*, *Patella*, *Acmæa*, etc.). In others the eggs contain a larger quantity of yolk and the free-swimming life is correspondingly reduced (*e.g.*, *Crepidula fornicata*, *Illyonassa*, etc.). In still others the eggs contain a yet greater quantity of yolk and the veliger stage is passed entirely within the egg capsules, the young escaping in practically an adult condition (*e.g.*, *Crepidula convexa* and *adunca*, *Urosalpinx*, *Sycotypus*, *Fulgur*, etc.).

A most curious and interesting method by which nutriment is supplied to the embryo is found in those gasteropods in which a large number of relatively small eggs is laid, only a few of which develop, the others being eaten as food by the developing embryos (*e.g.*, *Purpura*, *Buccinum*, *Fasciolaria*, *Neritina*, etc.).

For the purposes of the present study the only instances which we shall consider are those in which the food for the developing embryos is contained within the egg in the form of yolk. The early development of a considerable number of gasteropods is now well known, but most of these belong to that group having relatively small eggs. It has seemed to me worth while to compare with these the development of the largest gasteropod egg of which I have any knowledge, viz., that of *Fulgur carica*. The eggs of this species are about one-sixteenth of an inch in diameter; those of a closely allied form, *Sycotypus canalliculatus*, are about one-twenty-fifth of an inch in diameter. The eggs of *Fulgur* are about thirteen times the diameter and about 2,200 times the volume of the eggs of *Crepidula plana*, with which I shall particularly compare them. I have also studied several other species in which the eggs are intermediate in size between these two extremes, as shown by the following table:

Fulgur carica, ...	1,700 μ	in diameter.
Sycotypus canalliculatus, ...	1,000 μ	"
Crepidula adunca	410 μ	"
" *convexa*, ...	280 μ	"
" *fornicata*, ...	182 μ	"
" *plana*, ...	136 μ	"

The great difference in the sizes of these eggs is not due to a proportionate difference in all their constituents, viz., protoplasm as well as yolk, but almost entirely to the varying amounts of yolk which they contain, and this fact suggests the possibility of examining the influence of yolk upon development by comparing the development of one of the largest gasteropod eggs with one of the smallest.

A paper on the embryology of *Fulgur* was published by McMurrich (1886) more than twenty years ago. This paper deals in a general way with the development of this genus, questions of cell lineage and egg organization being at that time almost unknown. Further reference to McMurrich's work will be made throughout the course of this paper. A subsequent paper by the same author (1896) deals with the yolk lobe and centrosome of *Fulgur*. Some features in the development of this form were also considered by me (1897) in a paper dealing more particularly with *Crepidula*. Other than this the development of *Fulgur* has not been made the subject of any detailed study.

2. BREEDING HABITS.

For the sake of completeness I shall here mention some observations on the breeding habits of *Fulgur* and *Sycotypus*. In both of these forms the eggs are laid in peculiar disk-shaped or lenticular capsules which are attached in a series along a central cord. With the exception of the first few capsules formed, which are small and usually contain no eggs, each capsule contains from ten to twenty eggs embedded in a gelatinous substance which fills the capsules.

The method of forming the peculiar string of capsules characteristic of these genera is sufficiently interesting to merit a brief description. During the process of egg-laying, the foot of the female is contracted and partially withdrawn within the shell. A mucous-like secretion is then poured out from the nidamental organ through the folds of the partially contracted foot, and this secretion is applied to some solid object to which it adheres, or it is merely buried in the sand. In the case of *Fulgur* this first formed portion of the "string" is buried deeply in the sand; in certain specimens of *Sycotypus* which I kept in a wooden car, or float, the egg string was attached to the boards of the car. The first formed capsules on the string are small, far apart and contain no eggs. Later formed capsules increase in size and in the number of eggs contained in each, and successive capsules lie closer together on the string. During the process of laying the female is quiescent, and is usually buried beneath the surface of the sand, only the siphon protruding. The eggs, surrounded by the albumen and the secretion which forms the capsules, are poured out of the oviduct into a small cavity in the folded foot. Here the capsule-forming material comes into contact with the sea water and hardens, after which the capsule is released from the foot and another is formed. The average length of time taken by *Sycotypus* in forming a capsule is about three hours, and as the "egg string" may contain as many as seventy capsules, the egg laying may cover a period of several days.

The eggs of *Fulgur* are laid at Beaufort, N. C., during the months of May and June principally.[3] *Sycotypus* lays its eggs at Woods Hole late in August or early in September. The rate of development in these gasteropods is very slow. I can give no definite figures as to the length of the period of development, since the eggs which have been brought into the laboratory do not long continue to develop normally, but from such evidence as I have been able to gather I conclude that the development within the capsules occupies several months; indeed I have frequently found egg strings in midwinter with partially developed embryos, indicating that the eggs which are laid in the fall may not hatch until the following spring. As might be expected where eggs are so greatly laden with yolk, the entire embryonic and larval development is passed within the egg capsules and the young hatch in practically an adult condition.

3. METHODS AND MATERIAL.

The eggs of *Fulgur* and *Sycotypus* are very soft and are difficult to preserve. When removed from the very thick jelly in which they are enclosed within the capsules they usually flatten into thin disks under their own weight, or become otherwise distorted. There are no egg membranes except those formed by the surrounding gelatinous substance, and the thin pellicle of protoplasm which surrounds the egg is too weak to preserve the spherical shape or even to retain the yolk unless the eggs are floating in water or jelly. Since the jelly must in most cases be removed before the fixation of the eggs, special means must be employed to prevent them from bursting or becoming distorted. Many rapid fixing fluids, such as hot sublimate, alcohol and alcohol-acetic mixtures, cause the eggs to crack open, or even to split into fragments after they have been apparently well fixed. After experimenting with many fixing fluids, the only successful method of preservation which I have found, is to open one side of the capsule and place the capsule containing the eggs in the jelly in 20 per cent. formalin for an hour or two, and then shake the eggs out of the jelly into a mixture of 20 per cent. formalin and 70 per cent. alcohol, equal parts, afterwards transferring them to 80 per cent. alcohol. Unfortunately this method leaves shreds of the jelly adhering to the eggs, and as these shreds stain intensely they interfere with the study of the eggs, which are to be stained and mounted entire. To preserve eggs entirely free from the jelly I have found it advisable to slit open the jelly and allow the eggs to drop out into a tall jar of 20 per cent. formalin, or into a mixture of pure formalin (40 per cent.) and a saturated solution of corrosive sublimate, equal parts. If the jar is quite tall (at least eighteen inches) the eggs may be sufficiently hardened before they reach the bottom to preserve their spherical shape, especially if there is a layer of absorbent cotton at the bottom of the jar. After a preliminary hardening in this fluid the eggs may be transferred to other fixing fluids or to alcohol. These eggs were then stained in dilute Delafield's hæmatoxylin, and were mounted entire in the manner described by me (1897, 1902) for other kinds of eggs.

---

[3] I wish here to acknowledge my indebtedness to Hon. George M. Bowers, U. S. Commissioner of Fisheries, and to Dr. Caswell Grave, Director of the Fisheries Station at Beaufort, for the many courtesies extended to me while I was at the Beaufort Station.

II. CLEAVAGE.

1. THE UNSEGMENTED EGG.

I have made no attempt to study the phenomena of maturation and fertilization in these eggs since they are particularly unfavorable for such work, owing to the difficulties of fixation, already referred to, and to the great quantity of yolk, which make the eggs difficult to section.

The most striking feature of the unsegmented egg is the extremely small quantity of the nuclear and cytoplasmic material as contrasted with the yolk. The area of cytoplasm and the nuclear spindle shown in Plate XXIII, fig. 1, are but little larger than in the egg of *Crepidula*, though the entire egg of *Fulgur* is about 2,000 times the volume of the egg of *Crepidula*. The great increase in the size of the egg of *Fulgur* as compared with that of *Crepidula* is due almost exclusively to the increased quantity of the yolk.

2. FIRST AND SECOND CLEAVAGES.

It is one of the surprises connected with the development of this egg that although the yolk is so abundant the cleavage is yet holoblastic. When this work was first begun I had thought that the cleavage in this egg, which is larger than the eggs of many cephalopods, might show some resemblances to the meroblastic cleavage of the cephalopod egg. However, this is not the case, for the cleavages, at least as far as the 56-cell stage, are identically like those of *Crepidula* save for the enormous size of the macromeres. In the positions of the mitotic spindles, the direction and rhythm of division, and the shapes, relative sizes and number of the micromeres these two genera are practically identical. In the later development of *Fulgur* the yolk cells do not divide, although their nuclei do, and this may be an approach to the condition found in meroblastic eggs. Many characteristic differences appear between *Fulgur* and *Crepidula* in the later cleavages, and this fact is another evidence in favor of the view, which I have maintained elsewhere (Conklin, 1897, 1898), that the form of the early cleavage is more constant than that of the later cleavage; indeed the early cleavage pattern may be reckoned as one of the most conservative features in the development of any gasteropod.

The first cleavage divides the egg into two equal cells, one of which forms the anterior half of the future animal, the other the posterior half (fig. 2). At the close of this cleavage the nuclei and cytoplasmic areas rotate first in a dexiotropic and then in a læotropic direction, as in *Crepidula*, showing that the first cleavage is spiral in character.

During the first cleavage and again during the second a small lobe is formed at the vegetal pole, which again fuses with one of the macromeres at the close of the cleavage. This is the yolk lobe which has been observed in so many different animals. In forms in which the first two cleavages are equal this lobe is small, *e.g.*, in *Crepidula* it is quite inconspicuous, in *Fulgur* it is larger but still not more than one-sixth the diameter of the macromere; in forms in which the first two cleavages are unequal this lobe may be larger than the macromeres, *e.g.*, *Urosalpinx.* McMurrich mistook this lobe for the polar body, but afterwards (1896) corrected this mistake.

The second cleavage is at right angles to the first and divides the egg into right and left halves. It is not possible to affirm that the median plane of the future animal coincides precisely with the second cleavage plane, but it is evident that the two are very nearly, if not exactly, coincident. In this respect, as also in the læotropic position of the spindles and the relative positions of the daughter nuclei and spheres, this cleavage is similar to the corresponding one of *Crepidula.*

It is a surprising fact that a mitotic figure so small as that shown in fig. 1 can bring about the division of so large a cell. It does not seem possible that the mechanical influence of the amphiaster could cause so large a result, except as it may serve as a stimulus to other forces. It is doubtful too whether cytoplasmic movements of a vortical nature are sufficient to explain the division of a cell body in which there is so small an amount of cytoplasm and so large a quantity of yolk. The superficial layer of cytoplasm is so thin and weak that at first thought it would seem incapable of constricting and ultimately dividing this great cell. But although the factors named seem inadequate to produce so large a result it must be borne in mind that the yolk is relatively soft, almost fluid, and that a considerable quantity of cytoplasm is distributed through the yolk at this stage; furthermore the rate of division is extremely slow, many hours being necessary for the completion of each of the first two cleavages. These are the only equal divisions of the entire yolk which I have seen. Although the four macromeres give rise to many cells in the course of development they still constitute the chief volume of the embryo as late as the veliger stage (pl. XXVIII, fig. 31), thus showing that the yolk content of these cells has remained practically as in the 4-cell stage.

3. SEGREGATION OF THE GERMINAL LAYERS.

The third cleavage is very unequal and separates the first quartet of micromeres from the macromeres in a dexiotropic direction (pl. XXIII, fig. 4); these cells come to lie in the furrows between the macromeres, and their shape is consequently like the corresponding cells in *Crepidula*.

The fourth cleavage (fig. 4) separates the second quartet of micromeres from the macromeres in a læotropic direction. In anticipation of this cleavage the nuclei with the surrounding cytoplasm rotate læotropically until they lie in the upper left-hand corner of each cell, then the mitotic figures appear and the cleavage takes place precisely as in *Crepidula* and other gasteropods.

The first quartet cells then divide læotropically, giving off at their peripheral borders the "turret cells," and a little later the macromeres again divide in a dexiotropic direction, giving rise to the third quartet (fig. 5). Simultaneously the cells of the second quartet divide dexiotropically. These divisions are shown in figs. 5 and 6. As a result of these divisions twenty micromeres are formed, of which eight belong to the first quartet, eight to the second and four to the third (fig. 6). The entire ectoderm comes from these twenty cells. The micromeres are composed almost entirely of cytoplasm and are relatively free from yolk; in the macromeres the cytoplasmic areas surrounding the nuclei lie at the periphery of the cap of micromeres. The visible quantity of the cytoplasm has increased very considerably since the beginning of cleavage, as is readily seen by comparing figs. 1 and 6. This is probably due to the segregation of cytoplasm, originally distributed through the yolk, into the micromeres and cytoplasmic areas of the macromeres, rather than to the conversion of yolk into cytoplasm, since the yolk becomes less fluid and more firm during this period.

In *Fulgur* and *Sycotypus*, as in every other gasteropod so far studied, there are only three quartets of ectomeres. This is especially interesting in view of the enormous size of this egg, where we might expect, if anywhere, to find a much larger number of micromeres formed. McMurrich supposed that a large number of micromeres were budded off from the macromeres in *Fulgur*, and that the number of such micromeres varied with the size of the egg. Viguier (1898) and Fujita (1895) also reached the same conclusion, maintaining that the number of quartets formed in *Tethys* and *Siphonaria* is greater than three. This view is in my opinion wholly without foundation. Whether the egg be large or small three quartets of ectomeres are formed, no more and no less, and by the subdivision of the cells of these quartets all the ectoderm of the embryo is formed. In the cases cited by Viguier and Fujita it can readily be seen, from their own figures, that they have mistaken the subdivision of certain quartet cells for the formation of new quartets from the macromeres.

Since there are only three quartets of micromeres in *Fulgur*, and since these cells must overgrow the enormous yolk, it would seem reasonable to expect that the rate of subdivision of these quartets would be accelerated, as compared with forms in which the yolk is small. However this is not the case; the first quartet divides about the time of the formation of the second quartet, and the latter divides coincidently with the formation of the third quartet, just as in *Crepidula*. In much later stages the micromeres do divide more rapidly, but the early subdivisions of the three quartets is not more rapid in the case of this largest of gasteropod eggs than in the case of the smallest. In every respect except the size of the macromeres, the early cleavage of *Fulgur* is almost identically like that of *Crepidula* or any other prosobranch. In the number, position, shapes and relative sizes of the micromeres and in the positions and sizes of the nuclei and spheres there is the closest similarity between the largest and smallest of gasteropod eggs.

The next division is a highly characteristic one for annelids and mollusks, viz., the formation of the first member of the fourth quartet, 4*d*, by læotropic cleavage of the left posterior macromere.

After its formation it moves over into the furrow between macromeres *D* and *C*, as shown in pl. XXIV, fig. 8. This cell, 4*d*, is formed in advance of the other members of the fourth quartet and it contains a considerable quantity of yolk. In all of these regards it resembles the corresponding cell in all annelids and mollusks, with the exception of the cephalopods, in which the cell-lineage has been studied.

But the feature of particular interest in this cell is its destiny rather than its origin. In all annelids and mollusks, so far as known, the anterior portion of this cell gives rise to most of the mesoderm, while from its posterior portion the terminal part of the alimentary canal arises. This cell is therefore known as the mesentoblast. The history of this cell in *Fulgur* conforms entirely to what we know of it in other forms, as will appear later.

With the formation of the mesentoblast the segregation of the substances of the different germinal layers is nearly completed. I have not observed that the substances composing the mesentoblast differ visibly from those of the ectoblasts and entoblasts, except in the quantity of yolk which these different cells contain. But in *Limnæa, Physa* and *Planorbis* I have been able to distinguish the substance of the ectomeres from that of the mesomeres and entomeres before the first cleavage of the egg. In these fresh-water snails, therefore, there is a definite localization of the substances of the different germinal layers, and I have elsewhere shown reason for believing that it is the fact of this localization which determines that the ectoblast shall be separated in three quartets, and that the mesoblast shall arise from one cell of the fourth quartet. Since the segregation of the germinal layers occurs in exactly the same way in *Fulgur* as in these other gasteropods, it seems probable that there is here also the same type of localization of the germinal substances of the egg, and that the enormous accumulation of yolk in this egg has not altered in any fundamental way the localization of its various substances.

The other members of the fourth quartet, 4*A*, 4*B* and 4*C*, are not formed until the 50-cell stage (fig. 9). They are much larger than the cell 4*d* and contain a much greater quantity of yolk. Because of their great size I shall speak of them as the "secondary macromeres." These cells are purely entoblastic in character and give rise to a portion of the alimentary canal. In every one of these details they closely resemble the corresponding cells of *Crepidula.*

4. FURTHER HISTORY OF THE ECTOMERES.

The further history of the three quartets of ectomeres may now be followed through the development up to the time when they give rise to the first recognizable organs. About the time of the formation of the cell 4*d* all of the twenty micromeres divide so that forty micromeres are formed, as shown in fig. 8 (in this figure all the micromeres have divided or are dividing except the two posterior turret cells, which divide a little later, fig. 9). The directions of these divisions and the relative sizes of the daughter cells is the same as in *Crepidula,* and the time at which the divisions occur is also the same, except that the turret cells divide earlier in *Fulgur* than in *Crepidula.*

The latest stage to which I have been able to trace with certainty the lineage of every cell is shown in fig. 9. At this stage there are present the following cells:

Ectomeres	42
Mesentomeres	6
Entomeres	7
Total	55

Fig. 10 shows spindles in two cells of the second quartet, and when the divisions here indicated are completed in the four quadrants the number of cells of known lineage is raised to 59. As a result of this work on the early cleavage it may be affirmed with confidence that in *Fulgur* the lineage of every cell up to about the 60-cell stage is identically like that of *Crepidula.*

a. The First Quartet.—Ectodermal Cross.—In *Crepidula* and many other prosobranchs one of the most striking appearances in the early history of the ectomeres is the formation of the so-called "ectodermal cross." This cross is composed of all the cells of the first quartet, except the turret cells, and of four cells of the second quartet, one of which forms the tip or terminal cell of each arm of the cross. The center of the cross lies exactly at the animal pole, and one of the four arms is anterior, one posterior, one right, and one left. This ectodermal cross, composed of exactly the same cells as in *Crepidula,* is present in *Fulgur* also (fig. 9 *et seq.*). It is not, however, so distinctly marked off from the other micromeres as in *Crepidula,* and it is therefore much more difficult to trace its subsequent history. In pls. XXIV, XXV, figs. 8–16, the outlines of the cross are shown in heavy lines, the center of the cross is marked by a short cross line, and the tip of each arm is lightly stippled. In fig. 8 the cross consists of three cells in each quadrant, an "apical," a "basal" and a "tip" cell (the anterior tip cell is not yet formed). In fig. 9 the basal cells in the right and left arms have divided, and in fig. 10 the basal cell of the anterior arm has also divided. There are at this stage four apical cells and three cells in each arm of the cross, except the posterior one which contains but two. Later, as shown in fig. 11, the basal cell in the posterior arm also divides and at this stage the cross consists of sixteen cells. Still later the apical cells bud off small cells between the bases of the arms, the "rosette" cells of *Nereis* and *Crepidula* (fig. 12). In every one of these details of cleavage the history of the cross in *Fulgur* is like that in *Crepidula*. The latest stage in which I have been able to identify all the cells of the cross is one slightly later than the one shown in fig. 12. At this stage the cross consists of the following cells:

Apicals	4
Rosettes	4
Basals	7
Middles	7
Terminals	4
Total	26

In later stages the entire cross cannot be recognized with certainty. In figs. 13–16 the center of the cross, where the apical cells meet, can be located, and the terminal cells may be seen more or less clearly, but the other cells cannot always be surely identified. In fig. 13 the arms of the cross can be plainly seen, each consisting of four cells in a single linear series, as well as the four derivatives of the turret cells which lie between the arms of the cross, but the apical and rosette cells cannot be identified. In fig. 14 the cross is scarcely apparent at all, and no part of it can be identified with certainty. In fig. 15 a cross is shown with four cells in a series in each arm, just as in fig. 13. Fig. 16 shows a central plate of cells surrounded by heavy lines which probably represents the cross; the center of the apical cells may be recognized, more or less doubtfully, at the place marked by the cross line, and the terminal cells are indicated by the stipples. Midway between the terminal cells on each side of the plate is a group of four cells, also enclosed in heavy lines, which are the derivatives of the turret cells. There are thirty-six cells in the cross in this latest stage in which it is recognizable, eight of which are derived from the second quartet. Deducting these second quartet cells and including the turret cells, we find that there are forty-four cells of the first quartet shown in pl. XXV, fig. 16.

In *Crepidula* seven large cells of the anterior arm of the cross form a peculiar "apical cell plate," while a number of large ciliated cells derived from the posterior arm and the posterior turret cells form a large "posterior cell plate," which becomes the head vesicle of the larva. In *Fulgur* none of these structures are visible at any time, and although the cross may be recognized clearly enough in its earlier stages, it is quite evident that it is not so definite a landmark here as in *Crepidula*, where its cells may be identified with great certainty up to a stage where it consists of sixty-six cells. The turret cells in *Crepidula* grow to an enormous size and remain undivided up to a stage with more than 100 cells; in *Fulgur* they do not become larger than the other cells of the first quartet, and they divide twice before the 100-cell stage, as shown in figs. 13 and 16.

b. Second and Third Quartets of Ectomeres.—As far as I have been able to follow the lineage of these cells in *Fulgur*, it coincides precisely with that of the same quartets in *Crepidula*. The first subdivision of the second quartet in *Fulgur* is shown in fig. 7; the first division of the third quartet and the second of the second quartet in figs. 8, 9 and 10. As a result of the divisions indicated in these figures two cells of the third quartet and four of the second are formed in each quadrant of the egg. By the divisions indicated in fig. 10 one additional second quartet cell is formed in each quadrant, and by the spindles shown in fig. 12 still another cell is formed, making six second quartet cells in each quadrant of the egg shown in fig. 12. All the third quartet cells then divide, as indicated by the spindles in two of the cells in fig. 11, thus giving rise to four third quartet cells in each quadrant. In figs. 12–16 there are six cells of the second quartet and four of the third in each quadrant. Every one of these ten cells has its homologue in the corresponding stages of the egg of *Crepidula*.

This is as far as I have traced the lineage of all the cells of the second and third quartets, though individual cells of these quartets may be recognized in later stages (fig. 17). These cells of the second and third quartets are in general larger than those of the first quartet, which they completely surround. The third quartet cells are especially large, and in some cases (figs. 13, 15, 16) are quite as large as the derivatives of 4*d*.

In the stage shown in fig. 16 there are eighty-eight ectomeres as follows:

First quartet cells	44
Second quartet cells	28
Third quartet cells	16
Total	88

5. FURTHER HISTORY OF THE MESENTOMERES.

The cell 4*d*, formed at the 24-cell stage by læotropic division of the macromere *D*, undergoes dexiotropic cleavage into two equal cells, $4d^1$ and $4d^2$ (fig. 9). At first these two cells lie to the left of the median plane, but they later shift position so that the plane of division between them comes to coincide with the plane of bilateral symmetry. The next division of these cells separates an anterior protoplasmic portion from a posterior yolk-laden one (fig. 9), and this process is repeated at a slightly later stage (fig. 12), thus giving rise to six cells derived from the cell 4*d*, three on each side of the median plane.

Of these cells the two anterior ones on each side are small protoplasmic cells which lie wholly under the layer of ectodermal cells; these cells give rise to the mesoblastic band of each side. The posterior cells are much larger and contain a considerable quantity of yolk; they are only partially covered by the cap of ectodermal cells (fig. 12); they ultimately give rise to the terminal portion of the intestine. I have not followed in detail the cell lineage of these cells in later stages, owing in part to the fact that they lie close under the thin layer of ectoderm, and it is frequently difficult to distinguish one from the other in surface views. However it is sufficiently evident that these cells show fundamental resemblances in origin, history and destiny to the mesentoblast cells of *Crepidula* and other gasteropods. The "mesoblastic bands" of *Fulgur* do not present a single series of cells, as in some annelids and mollusks, but rather a broad, irregular band of cells which has the general form of the letter Y. The stem of the Y lies in the median plane behind the area of the shell gland, while its two branches diverge on each side of this structure (figs. 19–24).

Some distance in front of the anterior ends of these bands a few cells appear beneath the ectoderm, which are probably the homologues of the "larval mesoderm" of other forms (figs. 19–22). They are in close relation to the ectoderm cells which give rise to the cerebral ganglia. I have not followed these "larval mesoderm" cells until they give rise to mesodermal structures, but have classed them as mesoderm because they lie beneath the surface and resemble in appearance the cells of the mesodermal bands.

6. THE ENTOMERES.

Up to a stage when there are approximately fifty cells in the entire embryo the entomeres consist of the four macromeres only. These macromeres are all of about the same size, and they contain an enormous quantity of yolk as compared with the amount of protoplasm. Each macromere contains an area of cytoplasm relatively free from yolk, in which lie the nucleus and sphere. These cytoplasmic areas lie as near as possible to the animal pole and to the free surface of the cell; with the extension of the cap of ectomeres these cytoplasmic areas with their enclosed nuclei and spheres are not overgrown by the ectomeres, but they move out over the yolk at the margin of the ectodermal cap, and this position they retain until the blastoderm has overgrown much of the yolk (pl. XXVII, fig. 26).

At a stage of about fifty cells, the macromeres *A*, *B*, and *C* divide in a læotropic direction, giving rise to the remaining members of the fourth quartet, 4*A*, 4*B* and 4*C* (fig. 9). These cells are very large and full of yolk, and for these reasons I have called them "secondary macromeres." They come to lie in the furrows between the macromeres, and in this position they are partly overgrown by the advancing ectoderm and partly shoved before it. They serve as an excellent means of orienting the egg and embryo, since they are found at the right, left and anterior poles only, the posterior pole being occupied by the derivatives of the much smaller mesentoblast cell, 4*d*.

The secondary macromeres are purely endodermal in character. Although their nuclei subdivide (pls. XXV, figs. 17, 18, *et seq.*), their cell bodies usually remain undivided until the closure of the blastopore (pl. XXVIII, fig. 31). Throughout all of this period the entomeres consist of the four macromeres and of these three secondary macromeres. Even as late as the gastrula stage, shown in fig. 31, these are the only entoderm cells present except those derived from the cell 4*d*, which give rise to the terminal portion of the intestine (*In.*, figs. 29–31).

In *Crepidula* a fifth quartet of cells, which are purely entodermal in character, is formed from the macromeres about the time of the closure of the blastopore; and at the same time the cells 4*A*, 4*B* and 4*C* divide. These divisions have not been observed in *Fulgur*, but, with these exceptions, the origin, history and destiny of the entomeres of *Fulgur* are almost precisely like those of *Crepidula*. The four primary macromeres are similar in all regards, save only in bulk; the secondary macromeres arise at the same cell stage and behave in the same manner in these two genera. In *Crepidula*, as in *Fulgur*, these seven cells, together with the entoblastic derivatives of the cell 4*d*, constitute for a long period the whole of the entoblast.

With the stage represented by fig. 17, in which there are about 121 cells, the ectomeres become so numerous that their lineage cannot readily be traced. At a slightly later stage, regions of the blastoderm begin to differentiate into characteristic structures. We shall therefore consider this stage as the close of the cleavage and the beginning of the period of organ formation.

In conclusion we find that the cleavage of *Fulgur* resembles that of *Crepidula* in almost every detail. Such slight differences as do exist, as, for example, in the relative development of the "cross" in the two forms, are only such as frequently exist between other genera in which the eggs are about equal in size. In short the enormous size of the *Fulgur* egg, which has so greatly altered the relative proportions of cytoplasm and yolk, and of micromeres and macromeres, has not modified in the least the pattern of the cleavage. It has not modified the localization in the egg and the segregation in the cleavage of the substances of the germinal layers. In all of these respects the egg of *Fulgur* is typically like that of other gasteropods, and it shows no approach whatever to the condition found in the eggs of cephalopods.

7. RELATIVE SIZES OF NUCLEI AND CELLS.

The great quantity of yolk in the egg of *Fulgur* introduces some interesting cytological conditions. I have already called attention (p. 325) to the extremely small nuclear spindle in the first cleavage, and to the problem of how this little spindle and the small amount of cytoplasm around it can bring about the division of so great a cell body. Another, though related, problem is found in the relative sizes of the nucleus and cell body at different periods of the cleavage.

In all the early cleavages the nuclei and spindles are very small, even in the macromeres (figs. 1–6). Indeed the nuclei and spindles in the macromeres are not larger than those in the micromeres during the formation of the three quartets. After the formation of the third quartet (fig. 7) there is a relatively long resting period for all of the macromeres except *D*, consequently the nuclei in *A*, *B*, and *C*, fig. 8, have grown to nearly double the diameter of those in fig. 7. The macromere *D* divides before its nucleus becomes very large, giving rise to the mesentomere, 4*d*. About the 50-cell stage, fig. 9, the macromeres *A*, *B*, and *C* divide, giving rise to the secondary macromeres 4*A*, 4*B*, and 4*C*. The mitotic figures for this division are quite large and are proportional in size to the resting nuclei from which they arise.

From this stage onward the nuclei of all the macromeres enjoy a long "rest," during which they grow to double their maximum diameter at any previous stage (figs. 9–24). The greatest diameter of the nucleus during the 2-cell and 4-cell stages is about 40 μ; during the formation of the three quartets the nuclear diameter is not greater than this, but rather smaller; before the formation of the secondary macromeres (fig. 8) the diameter of the nuclei in *A*, *B*, and *C* is about 50 μ; at the close of their long rest and just before their next division (fig. 24) the nuclei are about 80 μ in diameter.

The diameter of the unsegmented egg is about 1,700 μ; that of each of the macromeres during the formation of the quartets about 970 μ; while in the stages after the formation of the fourth quartet it is somewhat less, say about 900 μ. Therefore, although the diameter of the cell body in these later stages is but little more than half that of the unsegmented egg, the nuclear diameter is twice as great as at any previous stage.

The ratio of the nuclear diameter to the cell diameter, the "Kernplasmarelation" (k/p) of Hertwig (1903), is about 1 : 40 in the unsegmented egg; about 1 : 24 in the 4-cell to 24-cell stages; about 1 : 20 just before the formation of the secondary macromeres; and about 1 : 12 at the close of the long resting period of the macromeres, shown in fig. 24.

In the micromeres of the 8–24-cell stages the ratio of the nuclear diameter to the cell diameter is about 1 : 5; and in the later stages, such as fig. 18, it rises to 1 : 3.

It seems perfectly evident from these figures that the absolute size of the nucleus is dependent not only upon the size of the cell, but also upon the length of the resting period; and by the same showing the length of the resting period is not determined primarily by the size of the nucleus. In all the earlier divisions of the macromeres of *Fulgur* the division comes on when the nucleus is relatively small (k/p = 1 : 24); in later stages it does not come on until the nucleus has grown to twice this size (k/p = 1 : 12).

In *Crepidula* I found (1902) that the size of the nucleus, chromosomes, centrosomes, spindles and asters was, in the last analysis, proportional to the volume of the cytoplasm, and I tried to show that the immediate cause of division was the growth of the nucleus to a point where the ratio of nucleus to cytoplasm exceeded a critical limit. This is certainly not the principal factor which brings on division in *Fulgur*; and the fact that mitosis may occur in one cleavage when the nucleus is relatively small and in another not until it becomes very large, suggests that the moment of division may be dependent upon some intrinsic condition in the nucleus or centrosome, rather than upon the ratio of nuclear size to cell size. Support is lent to this view by the phenomena of oogenesis; here we have in the germinal vesicle the largest nucleus in the entire life cycle, following upon one of the longest resting periods, while the second maturation division follows immediately after the first; here also the moment of division seems to depend upon intrinsic conditions in the cell.

In the division of the cell body *Fulgur* presents some interesting conditions. Attention has been called to the fact that although the spindle in the first cleavage is very small the entire egg divides. Equally surprising is the fact that in the divisions of the primary and secondary macromeres subsequent to the formation of the fourth quartet, the nucleus only divides while the cell body remains undivided (figs. 17–28). The cell bodies of these entomeres do not divide again until very late in development, if at all; even in figs. 34 and 35, in which the embryo shows many definitive structures, the furrow between two of the original macromeres can be seen running obliquely through the region below the head vesicle (*H. V.*).

It seems to me that the following explanation may be offered of this apparent anomaly. In the early stages of development the cell substance is quite fluid, as has been pointed out (p. 326), whereas in the later stages the substance of the macromeres becomes much more consistent. In the later stages of development the macromeres do not show the tendency to burst or to undergo distortion during fixation, which is so troublesome in the earlier stages; this is, I believe, due to the greater consistency of the yolk during the later stages. This same difference in consistency may explain also the curious fact that the entire cell divides in the early stages, whereas only the nucleus divides in later stages.

In this connection attention may be called to the fact that there is apparently a great increase in the quantity of cytoplasm in the later stages, as compared with the earlier ones (cf. figs. 1, 6, 25). This increased quantity of cytoplasm might be due to the liquefaction of the yolk and its transformation into cytoplasm, or it might be the result of the segregation into the ectodermal cap of cytoplasm originally spread through the yolk substance. It is probable that both of these processes occur, but in view of the increasing consistency of the yolk during the period of quartet formation, it seems very probable that most of the cytoplasm of the early stages was present as such, but in a diffused form, in the unsegmented egg.

III. ORGANOGENY.

It has been shown that there are but few and minor differences between *Fulgur* and *Crepidula* in the cleavage up to a stage of about 100 cells (fig. 16). After this stage the differences in development become more marked. The first notable difference is found in the number of micromeres; by repeated divisions of the three quartets of ectomeres these cells become very numerous in *Fulgur*, as shown in figs. 17, 18, *et seq.*, whereas in *Crepidula* the number remains relatively small. It is evident that the same cell stages in the two no longer represent similar stages in differentiation; for example, the shell gland is one of the first organs to appear, and is first visible in *Crepidula* when there are about 250 cells present (Conklin, 1897, fig. 74); while it first becomes visible in *Fulgur* when there are about 1,000 cells (fig. 23). The period of the closure of the blastopore is very different in the two; in *Crepidula* this occurs before any organs are visible (fig. 65), in *Fulgur* only after almost all the larval and many of the definitive organs are present (fig. 31).

Other striking differences between *Fulgur* and *Crepidula* are found in the form and character of the different organs and in the time at which they appear, but most of all in the method of formation of these organs and in their relation to the yolk.

1. OVERGROWTH OF THE YOLK AND CONCRESCENCE OF THE EMBRYO.

Owing to the fact that the entomeres are so large in *Fulgur*, organs begin to differentiate long before the closure of the blastopore, indeed while the blastoderm is still a small cap at the animal pole of the egg (figs. 23–26). The organ bases are here spread out as in a mercator's chart, whereas in *Crepidula* the organ bases do not appear until the blastoderm has overgrown the yolk and they occur over the sphere, so that only a few of them can be seen from a single point of view.

In the earlier stages of *Fulgur* the organ bases occur in the anterior half as well as in the posterior portion of the blastoderm (figs. 21–23); later by the very rapid growth of the median anterior portion of the blastoderm these organ bases are displaced laterally and posteriorly until they come to form a kind of germ ring, or crescent, along the posterior margin of the blastoderm (figs. 24–28). This germ ring contains the bases of all the future organs, whereas the rest of the blastoderm forms a kind of yolk sac.

The manner of the overgrowth is represented diagrammatically in the accompanying text figure. The outlines of the blastoderm in successive stages of the overgrowth are indicated by the numerals 1–8. By superimposing on the same figure these different stages of the overgrowth it is possible to see at a glance the character and relative amount of the movement of the different organ bases. Thus in 1, the center of the blastoderm is approximately the center of the animal pole; in front of this lie the cerebral ganglia (*cb*) and velar row (*v*), while the buccal ganglia (*bc*) lie at the right and left margins of the blastoderm. Posterior to the animal pole is found an organ complex which includes the shell gland, intestine, abdominal, parietal, pleural and pedal ganglia, and the otocysts.

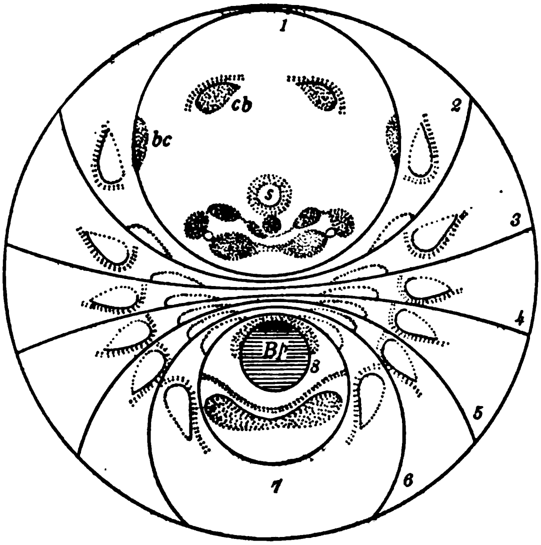


DIAGRAM illustrating the more important phases in the overgrowth of the yolk by the blastoderm in *Fulgur carica*. The outer circle represents the outline of the entire egg, and the smaller circles, numbered 1 to 8, represent the margins of the blastoderm in successive stages of the overgrowth. The egg is represented as seen from the posterior pole, and in order to bring all stages into one figure the arc through which the posterior margin of the blastoderm moves is represented as less than is actually the case. The outline of the blastoderm and the positions of the organ primordia in stage 1 correspond to figs. 25 and 26, plate XXVII; stages 2 and 3 correspond to fig. 27; stage 4 to fig. 28; stage 5 to fig. 29; stages 6 and 7 to fig. 30; stage 8 to fig. 31. In stage 1 the velar cells are represented as a double line of stipples anterior and lateral to the cerebral ganglia (*cb*); the buccal ganglia (*bc*) lie at the lateral margins of the blastoderm; immediately posterior to the shell gland (*s*) is the primordium of the intestine; on the left of this is the abdominal ganglion, and on each side of the latter are the parietal ganglia, the pleural ganglia, the otocysts and the pedal ganglia. In stages 2–7 the cerebral and buccal ganglia are represented in dotted outlines; in stages 1 and 8 they are stippled, as are the other primordia.

By the growth of the median anterior portion of the blastoderm, the cerebral ganglia and velar cells and the buccal ganglia are carried around to the successive positions indicated in the diagram, while the organ complex described above undergoes little movement. The diagram is faulty in that it shows too little movement of the organ complex of the posterior lip, and thus represents the final position of the blastopore too near the posterior pole, but it represents fairly well the relative movements of the different organ bases during the first four stages shown (1–4). Reference to the first and second cleavage furrows between the macromeres, shown in figs. 25–28, will show that the posterior margin of the blastoderm remains relatively fixed during this period, while at the same time the blastoderm extends forward and laterally; still later the blastoderm extends posteriorly also, and finally the blastopore closes at the vegetal pole (fig. 30).

2. APICAL INVAGINATION.

The first structure which appears in the course of development is an invagination of ectodermal cells just anterior to the animal pole and in the region of the anterior arm of the cross. This invagination is preceded by a broad depression of the blastoderm (fig. 18), and then the center of this depression becomes deeply cup-shaped (fig. 19), and finally tubular in form (fig. 20). The axis of this tubular invagination at first runs forward under the ectoderm; then it becomes perpendicular to the surface of the blastoderm; and subsequently it runs backward under the ectoderm, its opening being at its anterior end (fig. 20). The inner, blind end of this invagination lies near the anterior end of the polar furrow, and in some instances, though not in all, a few cells are separated from the invagination at this point. The anlagen of the cerebral ganglia are formed on the right and left of this invagination, but they do not appear to be derived from it. Similarly a few mesoderm cells, which probably correspond to the "larval mesoderm" of other mollusks, lie on each side of the invagination, though they are not derived from it (see the black nuclei in figs. 19 *et seq.*).

Subsequently this conspicuous and definite structure completely flattens out and disappears (figs. 21 *et seq.*), leaving not a trace behind. This fact seems so remarkable and the significance of the invagination is so problematical that I have devoted considerable time and effort to the study of it.

It is evident that this invagination is not merely the result of the sinking in of the blastoderm over a cavity in the yolk. There is frequently a cavity between the macromeres, but this invagination invariably lies anterior to this cavity, and in many cases it actually forces its way into the substance of the yolk. In some cases also a prominent evagination is formed instead of an invagination, showing that there is very active growth in this region of the blastoderm.

It seems to me probable that this invagination is a mechanical adaptation to secure a rapid extension of the anterior half of the blastoderm over the yolk. During the growth of the invagination the area of the blastoderm remains stationary, or actually grows smaller; as soon as it flattens out there is a very rapid increase in the area of the blastoderm (cf. figs. 20 and 21), especially of that portion lying anterior to the first cleavage plane. Coincidently with this increase in area the secondary macromere, 4*B*, begins to move forward in the furrow between the macromeres and the blastoderm rapidly extends forward. The remarkable extent of the growth of this anterior portion of the blastoderm may be seen by comparing the positions of the organ bases in figs. 19–30. In the earlier figures the shell gland and the two cerebral ganglia form the angles of an isosceles triangle, the base of which is directed forward (figs. 21 and 22); in later stages the ganglia separate more widely and the triangle becomes equilateral (figs. 23 and 24); still later the cerebral ganglia are separated so widely that they lie in line with the shell gland (figs. 27 and 28); and finally the cerebral ganglia again approach each other on the ventral side of the embryo (figs. 29 and 30). The greatest growth of the blastoderm takes place in the area between and in front of the cerebral ganglia, in the very region of the apical invagination, and it seems reasonable to suppose that the remarkable growth of this region is associated with the formation and subsequent flattening out of this structure.

McMurrich (1886) described at some length this invagination, and he compared it with a similar formation observed by Blochmann (1883) in *Neritina*, and by Sarasin (1882) in *Bythinia*. He says: "It seems very strange that an invagination so well marked as it is in *Neritina* and *Fulgur* should disappear and leave no trace of its existence, but so it seems to do." His further conclusion that it would be found to occur in most, if not all, of the prosobranch gasteropods has not been justified by later studies.

3. SHELL GLAND, SHELL AND MANTLE.

The shell gland is one of the earliest and largest of the developing organs, and is the one most instrumental in shaping the form of the embryo. It appears as an aggregation of ectodermal cells in the median plane posterior to the apical pole (figs. 21, 22). These cells, which are probably derived from the cell 2*d*, increase greatly in number and form a saucer-shaped depression (figs. 23–26, *Sh.*). This depression then becomes deeper and smaller in surface area (figs. 27, 28) and subsequently it evaginates in the manner characteristic of gasteropods, the margin of the gland forming a ridge, the mantle edge, while the center is covered by the cuticular substance which forms the earliest shell; beneath this shell is a very thin layer of ectoderm, in which the nuclei are few and far apart. The margin of the shell gland, or mantle edge, is at first circular in outline; then it becomes uniformly elliptical (figs. 29 and 30); finally it continues to extend in all directions, except where it comes into contact with the organ complex on the ventral side of the embryo; here the growth of the mantle edge is arrested, and consequently a notch in the developing shell and mantle appears here, which notch grows deeper as the mantle edge extends farther (figs. 31–36).

The area covered by the shell grows larger continually and the yolk appears to slip through the ring formed by the mantle edge, while at the same time the blastoderm and its organ anlagen are retained in front of this ring. In this way the yolk slips out of the anterior portion of the embryo, and the tension on the blastoderm being relieved in this manner, the latter is free to undergo the foldings necessary to form the head vesicle, velum, larval kidney and heart; at the same time the mantle edge, continuing to grow forward on the dorsal side, gives rise to the mantle fold and mantle chamber. On the ventral side the forward growth of the mantle edge is stopped by the organ complex (figs. 35, 36).

In the earlier stages of this overgrowth, the mantle edge and organ complex are apparently bilaterally symmetrical; in later stages it can be seen that the organs are more developed on the right side (left in ventral view) than on the left, and consequently the notch in the mantle edge is displaced from the median plane toward the right. In this way the assymetry of this gasteropod makes its appearance. Along the left side of the notch (right in ventral view) a cellular thickening of the blastoderm occurs near the mantle edge (figs. 34, 35, *CmM.*) which gives rise to the columellar muscle, while the shell formed along this portion of the mantle edge is the columella.

It is well known that in annelids the ectoderm of the trunk is derived from the ectomere *2d* (= X). In *Fulgur*, *Crepidula* and several other gasteropods, cells derived from the ectomere *2d* give rise to the mantle edge and fold and to the layer of cells covering the yolk under the shell. This fact suggests that the elongation of the embryo through the ring of the mantle edge may possibly be comparable to the elongation of the trunk of the annelid.

4. NERVOUS SYSTEM AND SENSE ORGANS.

In their earliest stages the organ bases may be recognized by the fact that the nuclei are closer together and the protoplasm stains more deeply than in other portions of the blastoderm. In the case of the nervous system certain of the ganglia are from the first clearly distinguishable, whereas some others in the organ complex posterior to the shell gland (fig. 23) cannot be distinguished until a later stage.

a. Cerebral Ganglia.—The group of cells which will form the cerebral ganglia appears at a very early stage. It is shown in figs. 19 and 20 on each side of the apical invagination, and closely connected with the group of cells lying beneath the superficial layer, which I have identified, somewhat doubtfully, with the "larval mesoderm" (fig. 21, *Lm.*). The cells which give rise to the cerebral ganglia arise in the region of the blastoderm, on each side of the anterior arm of the cross and in front of the transverse arms, probably from the "rosette" cells, exactly as in *Crepidula,* and they probably correspond in origin as well as destiny to the cells of the "cephalic neural plate" of *Nereis* (cf. Conklin, 1897, p. 110).

With the great growth of the anterior part of the blastoderm, which follows the flattening out of the apical invagination, these cerebral ganglia are carried laterally until they lie near the margin of the blastoderm (figs. 21, 22, *Cb.*). In figs. 23 and 24 they lie still nearer the margin, and their protoplasm and nuclei are somewhat more condensed than in the previous stage; the bases of all the organs now stain more deeply and they are all connected together by deeply staining protoplasm (figs. 23, 24). In all these stages the cerebral ganglia lie anterior to the middle of the blastoderm and are the anteriormost organs present. Later they are carried back until they lie in the posterior lip of the blastopore and at opposite ends of a transverse line which lies nearly in the plane of the first cleavage, and along which line most of the organ bases of the embryo are found (text fig. and figs. 27, 28). Still later, by a continuation of the movement already described, the cerebral ganglia are carried posterior to the first cleavage plane and to the other organs of the posterior lip, and they then approach each other on the ventral side of the embryo, and come to lie on each side of the blastopore (figs. 29, 30). This movement of the cerebral ganglia may be better described by pointing out their position with reference to the shell gland, which may be considered as a fixed point. In earlier stages the shell gland forms the apex of an isosceles triangle, the other angles being formed by the cerebral ganglia; then by the separation of the ganglia the triangle becomes equilateral, and still later the angle at the apex increases until the ganglia and the shell gland lie in one straight line; then by a continuance of the movement of the ganglia a triangle is formed on the opposite (ventral) side of the shell gland, which develops in the inverse order of that on the dorsal side (text figure). In the stages shown in figs. 30 and 31 the cerebral ganglia lie lateral and slightly posterior to the blastopore; in fig. 32 they lie on the sides of the blastopore, and a process is growing out from each toward the other in front of the blastopore; in fig. 33–36 they lie in front of the blastopore and are closely connected together by the cerebral commissure.

Along the posterior edge of each cerebral ganglion a leaf-like process is formed which is the tentacle (figs. 35, 36, *T.*), and at the outer end of the furrow which separates the tentacle from the blastoderm the eye appears (figs. 34–36).

b. Buccal Ganglia.—Lateral and slightly posterior to each cerebral anlage is a group of cells which gives rise to the buccal ganglion (figs. 22, 23, *Bc.*). These cells lie at the very periphery of the blastoderm and are probably derived from the ectomeres 2*a* and 2*c*. In the overgrowth of the yolk they accompany the cerebral ganglia, moving from the dorsal to the ventral side in the posterior lip of the blastopore (figs. 27–29), and finally coming to lie along the posterior border of the blastopore (fig. 30). When the blastopore closes the buccal ganglia of the two sides come into contact on the posterior side of the blastopore (fig. 31 and text figure).

c. Pleural, Pedal, Parietal and Abdominal Ganglia.—The other ganglia of the nervous system form part of the complex of organs lying between the shell gland and the posterior lip of the blastopore. The pleural ganglia lie on each side of the shell gland and on the lateral borders of the organ complex (figs. 23, 24); the pedal ganglia lie on the median side of the pleurals; close behind the shell gland are the groups of cells which give rise to the parietal and abdominal ganglia. The anlagen of these ganglia are not clearly separated at this time, and only in the later stages (fig. 28 *et seq.*) are they quite distinct. When the foot begins to appear (fig. 31) the pedal ganglia are included within it, and the pleural ganglia lie on each side of the foot, while the otocysts lie between the two (fig. 28 *et seq.*). The otocysts are here, as elsewhere, formed as an invagination of the superficial ectoderm. All the ectodermal organs of this region are derived from the cell 2*d*, with the possible exception of the pleural ganglia, which lie on the lateral borders of the organ complex and may therefore come, in part, from the cells 3*c* and 3*d*. In annelids the ectoderm of the trunk region and the ventral neural plate, which gives rise to all the nervous system posterior to the mouth, come from this same cell, 2*d*, the "first somatoblast." Lillie (1895) found the same condition among lamellibranchs.

Among the gasteropods this cell is not larger than the other members of the second quartet, but it is destined to give rise to all the important ectodermal organs posterior to the mouth. In this fact we have a striking illustration not only of the value of cell-lineage in comparative embryology, but also of the fundamental similarity of annelids, lamellibranchs and gasteropods.

The parietal and abdominal ganglia, with their connectives, are recognizable as thickenings of the blastoderm in a stage as early as fig. 28, but they are not clearly distinguishable until a later stage (figs. 30, 33, 34). In these figures the entire nervous system is easily recognizable. The cerebral ganglia are connected by the cerebral commissure anterior to the mouth, and they are united with the pleurals and pedals by the cerebro-pleural and the cerebro-pedal commissures respectively on each side of the œsophagus. The parietal ganglion of the left side lies almost directly behind the foot, and is connected with the pleural of the left side by the pleuro-parietal connective; the parietal of the right side lies on the right side of the foot and is connected with the pleural of that side, while the two parietals are connected together by the nerve loop which runs alongside of the intestine and near its middle bears the abdominal, or visceral, ganglion.

In these and the following stages (pl. XXVIII, figs. 35 and 36) the entire nervous system and the twisting of the parietal loop may be seen with diagrammatic clearness. This twisting, which is a part of the general movement toward the right of all the organs posterior to the foot, carries the left parietal ganglion to the right side of the foot, where it lies in contact with the right pleural, while the abdominal lies still farther toward the right and the dorsal side (fig. 36); at the same time the right parietal is carried up to the dorsal midline, where it lies on the dorsal side of the œsophagus (fig. 36). The entire nervous system, except the buccal ganglia, which are here hidden from view by the cerebrals, is shown in very nearly its definitive position in fig. 36. All of the six pairs of ganglia may be recognized in fig. 30, and four pairs are recognizable in fig. 23.

In all of these regards the nervous system of *Fulgur* shows fundamental resemblances to that of *Crepidula*. To one who had not observed the earlier stages in the origin of the cerebral ganglia it might seem that these ganglia, which lie so near the periphery of the blastoderm and so closely connected with the other organs of the posterior lip, could not possibly have arisen from cells of the first quartet anterior to the transverse arms of the cross. It is only by a study of the early history of these ganglia and their subsequent movements that one can determine that the cerebral ganglia, and indeed all the ganglia, arise in corresponding parts of the egg in *Fulgur* and *Crepidula*. Such a study shows that amidst all the differences in the mode of development of these ganglia, there is the most fundamental resemblance in their places of origin in the egg, and that therefore the localization of the germinal substances of the egg in the early cleavage cells follows the same pattern in these two genera.

5. THE VELUM.

The velum arises in close relation to the cerebral ganglia, and its place of origin furnishes another striking illustration of what are apparently fundamental differences between *Fulgur* and *Crepidula*, but which are in reality fundamental resemblances. In *Fulgur* the velum is first visible as a dense band of nuclei on the median and posterior sides of the cerebral ganglia (fig. 28, *V.*). These velar bands are widely separated from one another and are entirely disconnected. In later stages these velar bands more completely encircle the cerebral ganglia; each has somewhat the form of a parabola, one limb of which runs forward in the lip of the blastopore, while the other, which lies posterior to the cerebral ganglion, runs out towards the lateral regions of the embryo and there ends in the general blastoderm (figs. 29, 30). Subsequently, when the blastopore narrows and closes, the two limbs which run forward in its lips unite in front of the mouth (figs. 31, 32), while the lateral limb turns forward over the sides of that portion of the embryo which will become the head vesicle (figs. 33, 34). From the posterior side of each band there is given off a branch which runs across the embryo posterior to the mouth and there joins its fellow of the opposite side, thus giving rise to the post-oral band, while that portion of the velum which runs in front of the mouth is the pre-oral band. Both of these bands were observed and described by McMurrich (1886).

In subsequent stages the velum is drawn out into a prominent bilaminar fold (figs. 35, 36). This velar fold or lobe grows out to a very great size, much larger than is shown in fig. 36, the ciliated velar cells being borne around its edge, as in other prosobranchs. By the beating of these cilia it is probable that the embryos are able to move very slowly within the egg capsules, though they never swim freely, the young escaping from the capsules only after the velar lobe has been absorbed. The velum in *Fulgur* is not a highly sensitive and contractile organ, as in *Crepidula* and in many other forms; apparently, it cannot be retracted, even in its most fully developed stage, and it is needless to say that in the stage shown in fig. 36 there is no cavity into which the velum, the head or the foot could be retracted.

From the preceding account it is evident that during the later stages of its development the velum in *Fulgur* is much the same as in *Crepidula* or any other prosobranch. In its early history, however, it seems to be altogether different. It appears, as has been said, on the median and posterior side of the cerebral ganglia when these structures lie far apart in the posterior margin of the blastoderm (fig. 28); in *Crepidula* it first appears about the time of the closure of the blastopore as a transverse row of cells in front of the mouth. In *Fulgur* it consists of two bands of cells on opposite sides of the embryo, which are entirely separate from each other; in *Crepidula* it is a single structure continuous from side to side.

However, in spite of these striking differences, it can be shown, I think, that the velum has a similar origin in both of these genera. Traces of the velum in *Fulgur* may be found at a much earlier stage than that shown in fig. 28; thus in fig. 23 a row of what I take to be velar cells may be seen on the lateral and anterior sides of the cerebral ganglia (the nuclei of these cells are shown in heavy outline). The position of this row of cells indicates that it has arisen from the cells adjoining the ganglion on the anterior and lateral sides, and since the ganglia themselves come, in all probability, from the "rosette" cells, the velar cells must have come from the anterior "turret" cells and perhaps also from the terminal cells of the transverse arms of the cross. In short, the velar cells arise in *Fulgur* in the same region and probably from the same cells as in *Crepidula*. Here again, as in the case of the cerebral ganglia, there is fundamental agreement between *Fulgur* and *Crepidula* in the early and late stages in the development of the velum; it is only in those stages of the overgrowth of the yolk, which are undoubtedly highly modified in *Fulgur*, that we find striking differences between these two genera.

6. BLASTOPORE, MOUTH AND ŒSOPHAGUS.

By the very great growth of the blastoderm in the region of the apical invagination the anterior portion of the blastoderm rapidly surrounds the yolk, while its posterior margin, containing all the organ bases, remains relatively fixed in position near the animal pole (figs. 25–28). Subsequently this posterior margin also extends over the yolk to such an extent that the blastopore finally lies at the vegetal pole of the egg (fig. 30). The anterior lip of the blastopore is always composed of an extremely thin layer of cells, and this portion of the blastopore is usually circular in outline; the posterior lip of the blastopore is always composed of a thicker layer of cells than the anterior lip, and it is not circular but is compressed laterally, as shown in fig. 30.

The blastopore then narrows until it is almost if not entirely closed (figs. 31, 32), and an invagination of ectoderm cells occurs here, which is the stomodæum. The mouth is formed at the very place where the blastopore closes, and the œsophagus is formed by the elongation of the stomodeal invagination (fig. 36, *Œ.*).

In the closure of the blastopore and the formation of the mouth and œsophagus *Fulgur* completely resembles *Crepidula.*

7. INTESTINE AND OTHER PORTIONS OF THE ALIMENTARY CANAL.

The intestine is formed from enteroblast cells derived from the mesentomere 4*d*. These enteroblasts lie in the midline behind the shell gland and they constitute the stem of a Y-shaped group of cells, the branches of the Y being the mesodermal bands (figs. 22, 23). In its earliest stages the intestine is a round, densely staining group of cells, which lies at a lower level than surrounding portions of the blastoderm. After the posterior margin of the blastoderm has extended over to the ventral side of the embryo, the intestine lies between the margin of the shell gland behind and the visceral nerve loop and abdominal ganglion in front (figs. 28–30).

About the time of the closure of the blastopore the anlage of the intestine elongates toward the right (left in ventral view), becoming at first elliptical in shape (fig. 31) and then tubular (fig. 32 *et seq.*). Up to the time of this elongation it lies in the median plane; after this it, together with other organs posterior to the foot, moves to the right. The end of the intestine which remains nearest the midline is the anal end, though the anus does not form until a much later period; the end farthest to the right is the gastral end. The intestine lies just in front of and parallel with the edge of the shell; in fig. 32 its course is nearly transverse to the long axis of the embryo, the notch in the shell being at this stage very shallow. As the shell grows forward on the dorsal side more rapidly than on the ventral, this notch grows deeper, and the right edge of the shell becomes parallel with the long axis of the embryo (figs. 33–35); at the same time the intestine turns from a transverse to a longitudinal course. The visceral nerve loop accompanies the intestine in this movement, the abdominal ganglion always lying close to the mesial side of the intestine.

Finally the mantle cavity begins to form on the mesial side of the intestine, and the latter is thus carried into the roof of the mantle chamber, the anal end of the intestine remaining near the mantle edge, while the gastral end reaches to the deepest portion of the mantle chamber (fig. 36). In all of these details as to the formation of the intestine there is striking similarity between *Fulgur* and *Crepidula*.

In *Crepidula* the gastral end of the intestine opens into a lumen between the yolk cells, which give rise to the stomach and liver. In *Fulgur* I have observed no such lumen between the yolk cells, and while I think it probable that these cells form the stomach and liver in this animal, I have not traced their history far enough to speak with certainty upon this point. In *Fulgur* as in *Crepidula* the secondary macromeres lie at the inner end of the stomodæum (fig. 31), and they probably form that section of the alimentary canal immediately following the stomodæum.

8. THE FOOT.

The development of the foot is practically the same in *Fulgur* as in other prosobranchs. It appears as a thickening of that portion of the blastoderm lying between the otocysts behind, the blastopore in front, and the pedal ganglia on the sides (figs. 29 and 30). It is at first wide in transverse direction and narrow antero-posteriorly and it is bilobed, the two lobes being separated on the side of the blastopore by a groove in which the blastoderm remains thin (figs. 30 *et seq.*). The foot then gradually rises above the general level of the blastoderm until it becomes prominent, becoming about half as wide from side to side as in earlier stages (figs. 33, 34); at the same time it takes into itself the pedal ganglia and otocysts.

At the posterior end of the groove between the two lobes an invagination is formed which becomes the pedal gland (*P. G.*, figs. 33, 34). A transverse furrow on the surface of the foot then constricts off a smaller anterior lobe, the propodium, from a larger posterior one, the mesopodium and metapodium (figs. 35, 36).

9. LARVAL AND DEFINITIVE KIDNEYS.

Running laterally from the foot a ridge of cells develops on the right and left sides; this ridge lies some distance posterior to the velum, with which it is nearly parallel; its free border, or crest, becomes crenated (figs. 34, 35, *Ex.K.*). This is the larval or external kidney and, as in other prosobranchs, consists of large ectodermal cells which become loaded with nitrogenous waste substances. It is an interesting fact that the larval kidney of *Fulgur* does not appear until after the basis of the permanent kidney is present (figs. 32, 33), and its relatively small size seems to indicate that it is never an important excretory organ in this animal. I have not observed the destiny of these excretory cells in *Fulgur*, but in *Crepidula*, where they are relatively larger, they are ultimately pinched off and set free with their load of nitrogenous waste. In *Fasciolaria*, as Glaser (1905) has shown, the external kidneys become colossal organs, which are filled with nitrogenous waste substance. Here the great size of these organs is probably associated with high metabolism of nitrogen, the result in this case of the cannibalism of the embryo. The small size of these organs in *Fulgur* probably indicates low nitrogenous metabolism in the embryo.

The definitive kidney has no structural connections with the larval ones. It appears as a single structure near the gastral end of the intestine (figs. 32 *et seq.*), and a duct develops from it which runs along the right side (left in ventral view) of the intestine (figs. 34, 35). As a result of the invagination which forms the mantle chamber, both the kidney and the intestine come to lie in the roof of that chamber, and since the kidney lies near the edge of the mantle it undergoes little movement during this invagination; the intestine, which lies farther from the mantle edge, moves under the kidney during this invagination, and thus the relative positions of these two organs are interchanged, the kidney coming to lie on the left side of the intestine (fig. 36).

10. GILL AND HEART.

At its earliest appearance the gill lies on the anterior side of the kidney and in close contact with it (figs. 33, 34). Before the formation of the mantle chamber it is merely an aggregation of cells and shows no characteristic structure. After the formation of the mantle chamber it lies on the left side of the kidney and its anterior border becomes crenated, each of these lobules giving rise later to a tentacle-like process.

The larval heart (figs. 35, 36, *L.H.*) appears as a blister under the blastoderm at the right of the larval kidney (fig. 34). The walls of this vesicle contain smooth muscle cells and are pulsatile. I have observed no definite vessels leading to or from this heart, and it probably serves merely to keep lymph moving through irregular channels. In later stages the larval heart is carried up on to the dorsal side of the embryo behind the head vesicle and velum (fig. 36); this movement is a part of the general twisting of all the organs, which were originally posterior to the foot.

I have not observed the manner of origin of the definitive heart and have not been able to recognize its anlage, unless it may be the dark body between the reference letters *M* and *Pa* in fig. 36.

IV. CONCLUSIONS.

The development of the principal organs and organ systems of *Fulgur* has now been described, with especial reference to their cell-lineage and topographical relations. It has not been possible to trace the entire cell-lineage of any organ, owing to the very large number of cells which are present before organs appear, but it is possible to determine the quartet and usually the individual cell of the quartet from which any organ arises. In a few cases (cerebral ganglion, velum, intestine) organs may be traced back to individual cells of a stage much later than the quartet formation, but in no case is it possible to observe every division of the cells which enter into the formation of an organ. This lack of a complete knowledge of the cell-lineage is not peculiar to *Fulgur,* but is general among forms in which the cell-lineage has been studied, and it is not a great hindrance to the study of the localizations of morphogenetic substances and processes of the egg. If groups of cells which give rise to certain organs can be traced back to certain quartet cells, this is usually sufficiently detailed information as to the cellular origin of an organ. In *Fulgur* it is unusually easy to trace this connection between blastomeres and organs, owing to the fact that the organs appear while the blastoderm is still a flat plate.

Next to the resemblances between *Fulgur* and *Crepidula* in the early cleavages, there is no similarity between these forms more striking than that which is found in the cellular origin of homologous organs. Although the organs of these two genera may differ widely in size and early position, there is not a single instance in which there is any good reason for supposing that these organs have arisen from unlike cleavage cells of the early stages. On the other hand, there is the best of evidence that homologous organs in *Crepidula* and *Fulgur* arise from corresponding cells of the different quartets, and even from corresponding cells of much later stages (60-cell stage), even though in still later stages these organs may occupy widely different regions of the embryo, as in the case of the cerebral ganglia.

With regard to the later cleavages, it is not only impossible to follow the lineage of individual cells until they give rise to organs, but it is certain that the cells of these two genera cannot be individually compared, since there are many more cells in *Fulgur* at a given stage of differentiation than in *Crepidula.* For example, there are about 250 cells in the embryo of *Crepidula* at the time of the first appearance of the shell gland, in *Fulgur* there are about 1,000 cells, and in any comparison of the cells at this stage it must be remembered that in general four cells of the latter are equal to one of the former. Not only does the number of cells differ in corresponding stages of differentiation in these two forms, but even in the same species there are variations and irregularities in the later cleavages which are not present in the earlier ones. When these irregularities of the later cleavages are compared with the invariable features of the early ones, such as the segregation of the ectoderm in three quartets of cells and the origin of the mesoderm of the trunk region from one cell of the fourth quartet, it is impossible to avoid the conclusion that the early cleavages are of greater morphogenetic value than the later ones. Furthermore, the conditions found in *Fulgur*, as compared with those in *Crepidula*, show that neither the presence of a large amount of yolk in the egg before cleavage, nor the modifications of the later stages, due to the presence of this yolk, affect the localization of the morphogenetic materials and processes in the earlier stages. The conclusion seems to be justified that the type of this localization is a more general and fundamental character than the form of gastrulation, or any other relatively late process in the ontogeny.

In *Fulgur* as in many other animals the egg shows polar differentiation while it is still in the ovary, and even before yolk formation begins. The yolk is laid down in all parts of the egg, and before maturation one pole is not noticeably richer in protoplasm than another. With the maturation of the egg the segregation of the protoplasm and yolk begins, most of the protoplasm passing to one pole and becoming the future ectoderm, while the yolk remains at the opposite pole and with a relatively small amount of protoplasm gives rise to the endoderm. In the first two cleavages this yolk is distributed equally to the four quadrants of the egg, and since the blastopore forms at the vegetal pole, the ectoderm must extend equally (but not synchronously) over the yolk in all directions. In the early stages of this overgrowth the anterior portion of the blastoderm extends more rapidly than the posterior portion, and by this means the cerebral and buccal ganglia are brought into close relation with the other organs present in the posterior margin of the blastoderm.

In many animals the yolk is not distributed equally to the four quadrants of the egg (*e.g.*, *Urosalpinx*, *Nassa*, *Dentalium*, *Chœtopterus*, etc.), and wherever this is the case a "yolk lobe" of considerable size is present. When one macromere is much larger than the others a large yolk lobe is attached to it; when the cleavage is approximately equal the yolk lobe, if present, is small. Crampton (1896) has shown that the removal of the yolk lobe causes the absence of mesoderm in *Illyonassa*; and in his beautiful "Studies on Germinal Localization" Wilson (1904) has found that when the polar lobe of *Dentalium* is removed the resulting larva lacks the post-trochal region and probably also the mesodermal bands. Both of these authors found that following the removal of the lobe the four quadrants of the egg were equal in size. It cannot be assumed that the size of the lobe is proportional to the size of the somatoblasts 2*d* and 4*d*, or to the size of the post-trochal region, or to the quantity of mesoderm which is formed later. In *Fulgur*, *Crepidula* and all other cases in which the quadrants are nearly equal in size, the yolk lobe is much smaller than the post-trochal region or the mesentomere 4*d*, or even the mesodermal bands; while in *Illyonassa*, *Tritia*, and other forms in which one of the macromeres is much larger than the others, the yolk lobe is much larger than the cell 4*d*. On the other hand the lobe is usually associated with unequal division of the yolk, and its size is proportional to that inequality. Therefore I believe that the real significance of the yolk lobe is to be found in its relation to equal and unequal cleavage.

Since the blastopore forms at the vegetal pole in all gasteropods, so far as known, unequal division of the yolk must lead to unequal growth on the part of the blastoderm; if the posterior macromere is large the overgrowth must be greater in a posterior direction than in any other; if the anterior macromere is larger than the others, as in certain opisthobranchs, the blastoderm must grow more anteriorly than posteriorly. When all the macromeres are equal the overgrowth must take place equally in all directions, though the case of *Fulgur* shows that it may be accelerated at one time in one portion of the blastoderm and at another time in another portion.

The most striking difference between *Fulgur* and other gasteropods is found in the manner of this overgrowth, and consequently in the manner in which the different organ bases are carried from their point of origin to their definitive positions in the embryo. In both the point of origin and the definitive position of the various organs there is fundamental agreement between *Fulgur* and *Crepidula*, but in the manner of passing from their point of origin to their final position there is marked dissimilarity. In *Crepidula* the region of greatest growth of the blastoderm lies on the dorsal side between the anlagen of the cerebral ganglia and the shell gland; in *Fulgur* it lies anterior to the cerebral anlagen. In *Crepidula*, as a result of this condition, the cerebral ganglia are carried forward around the anterior end of the embryo until they come to lie anterior to the mouth on the ventral side. In *Fulgur* the same ganglia are carried backward around the yolk until they finally reach the same position on the ventral side of the embryo.

It has been customary hitherto to regard such peculiarities of the embryo as primary, and the associated peculiarities of cleavage stages as secondary adaptations to these later appearing peculiarities; but this puts the cart before the horse. The adaptations of cleavage are adaptations to conditions pre-existing in the egg, and not adaptations to conditions which appear later in the embryo or adult. Given the enormous yolk of *Fulgur* or the small yolk of *Crepidula plana*, and the embryo must adapt itself to these conditions of the egg; or, in other words, the earlier conditions in ontogeny stand in the relation of cause to the later conditions, and not the reverse.

When I first observed in *Fulgur* the great modifications in the location of organ bases, which is unlike anything hitherto described, and found, for example, that the cerebral ganglia and velum were located in the posterior lip of the blastopore, I thought, for a time, that here was a new pattern of germinal localization, and that the generalization that homologous structures always come from homologous regions of the egg had broken down. Further study has shown that this is not the case, and that the great modifications in the location of embryonic organs in *Fulgur* are not primary but secondary, while the localization pattern in the early cleavages is the same as in other gasteropods.

Hence I regard the case of *Fulgur* as a triumph for the method and doctrine of cell-lineage. Those who see in this method only 'the counting of cells,' 'mitotic book-keeping,' 'the drudgery of dull minds,' have missed the whole point and significance of this method, which is not to name every cleavage cell, but to determine in what areas of the egg certain morphogenetic processes are located. To know that such processes may be localized in the egg is valuable information, even though the pattern of this localization should differ for every animal; but to have discovered that through all the multifarious modifications which are found in the embryos and adults of great animal classes, such as the gasteropods or annelids, this same pattern runs unchanged,—this is illuminating.

SUMMARY.

1. The eggs of *Fulgur carica* are among the largest of gasteropod eggs, their relatively great size being due almost exclusively to the great quantity of yolk which they contain. These eggs are thirteen times the diameter and about 2,000 times the volume of those of *Crepidula plana*, with which particularly they are compared.

2. The cleavage of the egg of *Fulgur* is, cell for cell, like that of *Crepidula* up to the 56–60-cell stage, the only difference being in the

relative sizes of the macromeres in these two genera. Some of the individual features in which the cleavage of *Fulgur* resembles that of *Crepidula* are the following:

a. The direction, rhythm and quality of each cleavage is the same.

b. The first and second cleavages are equal; the first is transverse to the median plane of the embryo, the second coincides with that plane; a small yolk lobe is formed during each of these cleavages.

c. The ectoderm is segregated in three quartets of micromeres.

d. The first formed member of the fourth quartet, 4*d*, is the mesentomere; its anterior portion gives rise to the mesodermal bands and its posterior portion to the intestine.

e. The remaining members of the fourth quartet, viz., 4*A*, 4*B*, 4*C* are entomeres and give rise to all portions of the alimentary canal and its outgrowths, save the intestine and stomodæum.

f. The first quartet of ectomeres forms a "cross", with its centre at the apical pole and with anterior, posterior, right and left arms. Between the arms are the "turret" cells, and between these and the apical pole are the "rosette" cells; all of these cells are derived from the first quartet, except the "terminal" cells of the arms which are derived from the second quartet. The cross is formed of exactly the same cells in *Fulgur* as in *Crepidula,* but it is neither so distinct in form nor so persistent.

g. The second and third quartets surround the first and are composed of cells which divide in the same order and direction as in *Crepidula.*

3. In later cleavages many more ectoderm cells are formed in *Fulgur* than in *Crepidula* at corresponding stages of differentiation.

4. The overgrowth of the yolk is highly peculiar in *Fulgur.* By very great extension of the anterior half of the blastoderm, while the posterior half remains relatively fixed, all the organ bases are carried to the posterior margin of the blastoderm, where they form a kind of germ ring. Subsequently the posterior margin also moves over the yolk, so that the blastopore is finally formed at the vegetal pole.

5. Before the extension of the anterior portion of the blastoderm an apical invagination of ectoderm cells is formed in the region of the anterior arm of the cross. This invagination subsequently flattens out and completely disappears. Its function seems to be connected with the rapid extension of this part of the blastoderm.

6. The cerebral ganglia arise on each side of the apical invagination, probably from the anterior "rosette" cells. The velar cells arise around the outer margins of the ganglia, probably from the anterior

"turret" cells. By the rapid growth of the anterior portion of the blastoderm these organ bases are forced far apart and posteriorly until they come to lie in the posterior margin of the blastoderm, and by a continuation of this movement they are carried around to the ventral side of the embryo, where the two halves of these organs approach each other and finally unite in front of the mouth.

7. The paired buccal ganglia arise lateral to the cerebral and probably from derivatives of 2*a* and 2*c*; they extend over the yolk in the same manner as the cerebrals and finally concresce on the ventral side of the embryo posterior to the mouth.

8. All other organs (shell gland, pedal, pleural, parietal and abdominal ganglia, foot, intestine, kidney, gill, heart, etc.) arise from the median posterior portion of the blastoderm, and chiefly, if not entirely, from the two "somatoblasts," 2*d* and 4*d*. The bases of all of these organs, except the last three, appear when the blastoderm is still a small plate, and in the case of paired organs they are from the first connected across the median plane and do not, therefore, undergo concrescence. The origin of these post-oral organs from the two somatoblasts is strikingly like the condition found in annelids, and the elongation of the body of the gasteropod through the shell gland is similar to the elongation of the body of the annelid.

9. All homologous organs arise from corresponding cleavage cells in *Fulgur*, *Crepidula*, and probably all other gasteropods. Great increase in yolk does not modify the type of germinal localization, though it does profoundly modify gastrulation and later stages.

10. This indicates that germinal localization in the egg and in the cleavage stages is more fundamental and primitive than are the later processes of ontogeny.

CYTOLOGICAL.

11. During quartet formation the nuclei of the macromeres divide at relatively short intervals and they remain relatively small, the ratio of maximum nuclear diameter to cell diameter ("Kernplasma-relation" K/p) being 1 : 24. In later stages the resting period of the nucleus is very long and the ratio rises to 1 : 12. The size of the nucleus is therefore dependent not only upon the size of the cell, but also upon the length of the resting period. There is in this species no fixed ratio of nuclear size to cell size, and the cause of cell division cannot be found in the maintenance of a constant ratio.

12. After the formation of the fourth quartet the macromeres do not again divide, although the nuclei do. In the first and second cleavages the macromeres divide equally; this apparent anomaly is

probably due to the fact that cytoplasm is distributed through the yolk in the early stages and is lacking in the later ones. This suppressed cleavage of the yolk in the later stages is an approach to the condition found in meroblastic eggs.

REFERENCES.

No attempt is made in this paper to review recent important contributions on cell-lineage, foremost among which must be mentioned the important memoirs of Robert (1903) on Trochus, Wierzejski (1905) on Physa, and Carazzi (1906) on Aplysia. The papers here listed are those mentioned in the body of this work.

BLOCHMANN, F. 1882. Ueber die Entwicklung der *Neritina fluviatalis*, *Zeit. wiss. Zool.*, XXXVI.

BROOKS, W. K. 1879. The Acquisition and Loss of Food Yolk by Molluscan Eggs, *Studies from Biol. Lab. Johns Hopkins University*, Vol. I.

CONKLIN, E. G. 1897. The Embryology of Crepidula, *Jour. Morph.*, XIII.

——. 1902. Karkokinesis and Cytokinesis in the Maturation, Fertilization and Cleavage of Crepidula, *Jour. Acad. Nat. Sci. Phila.*, Vol. XII.

CRAMPTON, H. E. 1896. Experimental Studies on Gasteropod Development, *Arch. Entwm.*, III.

FUJITA, T. 1895. Preliminary Note on the Mesoderm Formation of Pulmonata. *Zool. Mag. Tokio*, Vol. VII.

GLASER, O. C. 1905. Ueber den Kannibalismus bei *Fasciolaria tulipa* (var. *distans*) und deren larvale Excretionsorgane, *Zeit. wiss. Zool.*, LXXX.

HERTWIG, R. 1903. Ueber Korrelation von Zell- und Kerngrosse und ihre Bedeutung für die geschlechtliche Differenzierung und die Theilung der Zelle, *Biol. Centralb.*, XXIII.

LILLIE, F. R. 1895. The Embryology of the Unionidæ, *Jour. Morph.*, Vol. X.

McMURRICH, J. P. 1886. A Contribution to the Embryology of the Prosobranch Gasteropods, *Studies from Biol. Lab. Johns Hopkins University*, Vol. III.

——. 1896. The Yolk Lobe and Centrosome of *Fulgur carica*, *Anat. Anz.*, XII.

MORGAN, T. H. 1893. Experimental Studies on Teleost Eggs, *Anat. Anz.*, VIII.

SARASIN, P. 1882. Entwicklungsgeschichte der *Bythinia tentaculata*, *Arb. Zool. Inst. Würzburg*, VI.

VIGUIER, C. 1898. Contribution a l'étude du development de la *Tethys fimbriata*, *Arch. Zool. Exp.*, 3, XVI.

WILSON, E. B. 1904. Experimental Studies on Germinal Localization: I. The Germ Regions in the Egg of Dentalium, *Jour. Exp. Zool.*, I.

REFERENCE LETTERS.

A, *B*, *C*, *D*, Macromeres.
1a, *1b*, etc., First quartet cells.
2a, *2b*, etc., Second quartet cells.
3a, *3b*, etc., Third quartet cells.
4d, Mesentoblast.
4A, *4B*, *4C*, Secondary macromeres.
A.I., Apical invagination.
Bp., Blastopore.
Cb., Cerebral ganglion.
Bc., Buccal ganglion.
Pl., Pleural ganglion.
Pd., Pedal ganglion.
Pa., Parietal ganglion.
Ab., Abdominal ganglion.
E., Eye.
G., Gill.
H.V., Head vesicle.
I., Intestine.
K., Kidney.
Ex.K., Larval kidney.
L.H., Larval heart.
L.M., "Larval mesoderm."
M., Mantle.
Ms., Mesodermal bands.
O., Otocyst.
P.G., Pedal gland.
Sh., Shell gland.
T., Tentacle.
V., Velum.
Cm.M., Columellar muscle.
Oc., Œsophagus.

Description of Plates XXIII—XXVIII.

All the figures were drawn at the stage level with the aid of the camera lucida; plates XXIII, XXVII and XXVIII under Zeiss Apochromatic Obj. 16 mm., Oc. 4; plates XXIV, XXV and XXVI with Obj. 8 mm., Oc. 4. The figures of the former plates are therefore magnified 62 diameters; of the latter, 124 diameters. In the process of reproduction the figures were reduced a little more than half, so that as they appear on the plates they represent a magnification of about 30 diameters and 60 diameters respectively. All the drawings are of eggs or embryos of *Fulgur carica*, fixed, stained and mounted as described in the first section of this paper.

Plate XXIII.—Entire eggs; 1-cell to 24-cell stages.

Fig. 1.—Unsegmented egg, showing first cleavage spindle and surrounding cytoplasm.

Fig. 2.—Two-cell stage, showing læotropic turning of cytoplasm. nuclei and spheres preparatory to the second cleavage.

Fig. 3.—Four-cell stage; the positions of nuclei and spheres indicates that the transverse furrow is here the second one.

Fig. 4.—Eight-cell stage, showing first quartet of micromeres which have turned in a dexiotropic direction; in two of the macromeres are nuclear spindles for the formation of the second quartet, which arises in a læotropic direction.

Fig. 5.—Sixteen-cell stage. The first quartet has divided læotropically, giving rise to the "apical" cells centrally and the small "turret" cells peripherally. The second quartet cells are dividing in two quadrants, and the third quartet cells are arising dexiotropically from the micromeres.

Fig. 6.—Twenty-four-cell stage, resulting from the completion of all the divisions initiated in the preceding figure.

Plate XXIV.—Blastoderms during the earlier stages of cleavage.

Fig. 7.—Same stage as shown in fig. 6, but magnified twice as much; twenty micromeres and four macromeres; the separation of the ectoderm from the macromeres is now complete.

Fig. 8.—Forty-five-cell stage. Every cell of the preceding stage has divided, or is dividing, except the macromeres *A*, *B* and *C*. The "apical" cells have given rise to the "basals" of the arms of the cross; one second quartet cell in each quadrant has produced the "tip" cell (stippled) of each arm; the third quartet cells are dividing læotropically, and the macromere *D* has produced the mesentoblast cell, 4*d*.

Fig. 9.—Fifty-five-cell stage. All divisions initiated in the preceding figure are here completed, and in addition the basal cells of the transverse arms of the cross have divided. The mesentoblast (4*d*) has given rise to six cells (two large and four small ones), and the macromeres *A*, *B* and *C* have given off the other members of the fourth quartet, viz., 4*A*, 4*B* and 4*C*.

Fig. 10.—Fifty-six-cell stage. Similar to the preceding, but showing the basal cell of the anterior arm of the cross divided and spindles in some of the cells derived from the second quartet. The cross is shown in heavy outline; there are three cells in each of the arms except the posterior one. The centre of the cross is here, and elsewhere, marked by a cross line, the "tip" cells of the arms, by stipples.

Fig. 11.—Irregular 81-cell stage; derived from preceding stage by division of the basal cell of the posterior arm, of eight cells of the third quartet, of eight cells of the second quartet, and of eight turret cells.

Fig. 12.—Eighty-five-cell stage; derived from the preceding by the completion of all divisions there indicated and by the subdivision of the basal and middle cells in the transverse arms of the cross.

PLATE XXV.—Blastoderms, showing later stages of cleavage.

Fig. 13.—Another 85-cell stage, consisting of 72 ectomeres, 6 mesentomeres and 7 entomeres.

Fig. 14.—Stage with 111 cells; 98 ectomeres, 6 mesentomeres and 7 entomeres. This is a more advanced stage than the two following ones.

Fig. 15.—Stage with 89 cells; 76 ectomeres, 6 mesentomeres, 7 entomeres.

Fig. 16.—Stage with 104 cells; 91 ectomeres, 6 mesentomeres, and 7 entomeres.

Fig 17.—Stage with 121 cells; 104 ectomeres, 10(?) mesentomeres and 7 entomeres.

Fig 18.—Stage of about 320 cells. The ectomeres are very numerous and are somewhat depressed below the general level in the region anterior to the apical pole. The nuclei of the secondary macromeres, 4*A*, 4*B* and 4*C*, are dividing.

PLATE XXVI.—Blastoderms, from the time of formation of the apical invagination to the appearance of the primordia of definitive organs. All nuclei and cell boundaries, where shown, were drawn with the camera lucida, so that their number and location are fairly accurate.

Fig. 19.—Stage of approximately 512 cells. The apical invagination (*A.I.*) is a deep pit just anterior to the animal pole. The mesentoblast (4*d*) has given rise to the mesodermal bands (*Ms.*).

Fig. 20.—Stage slightly more advanced than the preceding, showing the apical invagination as a tubular ingrowth, with its opening near the anterior edge of the blastoderm; the nuclei of the secondary macromeres (4*A*, 4*B* and 4*C*) are dividing a second time.

Fig. 21.—Stage immediately after the flattening out of the apical invagination, only a trace of which is here shown. On each side of the latter is a group of cells, lying beneath the surface of the blastoderm, and represented with solid black nuclei, which are probably "larval mesoderm" cells (*L.M.*); the superficial group of cells in this region ultimately gives rise to the cerebral ganglion (*Cb.*), while the aggregation of cells lying between and in front of the mesodermal bands (*Ms.*) is the primordium of the shell gland. Two of the secondary macromeres (4*A* and 4*C*) have, exceptionally, divided.

Fig. 22.—In this and the following figures all traces of the apical invagination have disappeared; in other respects this figure is similar to the preceding one.

Fig. 23.—Stage of about 1,000 cells, in which the shell gland is plainly visible as a disk of cells, slightly depressed in the middle, and in which the primordia of the cerebral (*Cb.*), buccal (*Bc.*), pleural (*Pl.*), and pedal (*Pd.*) ganglia and of the intestine (*In.*) are recognizable as groups of cells. The row of nuclei on the outer side of each cerebral ganglion probably represents the primordium of the velum, while the more deeply staining area connecting the two cerebral ganglia across the apical pole probably corresponds to the "cephalic neural plate" of annelids. The nuclei of the secondary macromere (4*B*) are dividing a third time, but the cell body remains undivided.

Fig. 24.—Stage in all regards similar to the preceding, except that the blastoderm has grown larger. The nuclei in the macromeres have here reached their maximum size (cf. the nuclei of the same cells in figs. 7 and 10).

LATE XXVII.—Entire eggs, showing stages from the appearance of organ primordia to the completion of the overgrowth of the yolk.

Figs. 25 and 26.—Stages similar to the one shown in fig. 24. The primordia of different organs are indicated by the closely stippled areas, the stipples representing nuclei.

Fig. 27.—Stage in which the blastoderm has extended through the growth of its anterior portion, over about one-third of the yolk. The organ primordia are confined to a small area in the posterior margin of the blastoderm. The nuclei of the macromeres are dividing.

Fig. 28.—Stage in which the blastoderm has overgrown about one-half of the yolk. Primordia of the following organs are shown: Cerebral ganglia (*Cb.*), velum (*V.*), buccal ganglia (*Bc.*), pleural ganglia (*Pl.*), pedal ganglia (*Pd.*), otocysts (between pleural and pedal ganglia), intestine (*In.*), parietal ganglia (on each side of intestine), and shell gland.

Fig. 29.—Stage in which about two-thirds of the yolk has been overgrown. In addition to the organ primordia already named, the foot is also visible between the two pedal ganglia.

Fig. 30.—Final stages in the overgrowth of the yolk. The blastopore (*Bp.*) is a relatively small area of yolk at the vegetal pole, still uncovered by the blastoderm. The secondary macromeres (4*A*, 4*B* and 4*C*) lie in the lips of the blastopore, and while they contain many nuclei their cell bodies are still undivided. The velum has begun to extend forward around the anterior side of the blastopore, while the buccal ganglia (*Bc.*) lie in its lateral borders. All the organ primordia named above are plainly visible, and in addition the abdominal ganglion may be seen on the left of the intestine.

Plate XXVIII.—Entire embryos, from the closure of the blastopore to the formation of the larva.

Fig. 31.—Embryo showing the blastopore very small. The margin of the shell gland has extended widely, while the area within this margin is covered by the embryonic shell (*Sh.*).

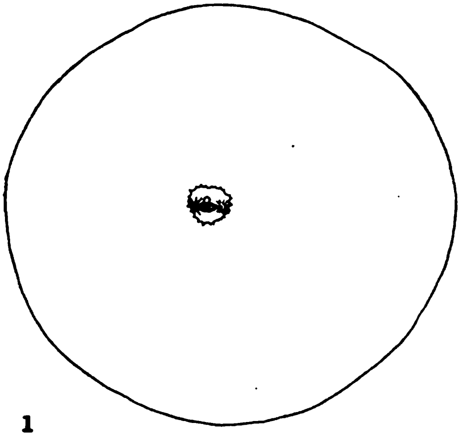
Fig. 32.—Stage showing the closure of the blastopore and the establishment of the pre-oral and post-oral velar bands The margin of the shell gland shows a deep notch posterior to the intestine, which grows more pronounced in later stages. The two cerebral ganglia are approaching each other in front of the blastopore. The primordium of the permanent kidney (*K.*) is recognizable.

Fig. 33.—Older stage, in which nearly one-half of the embryo is covered by the shell. The cerebral ganglia are united by a commissure in front of the mouth.

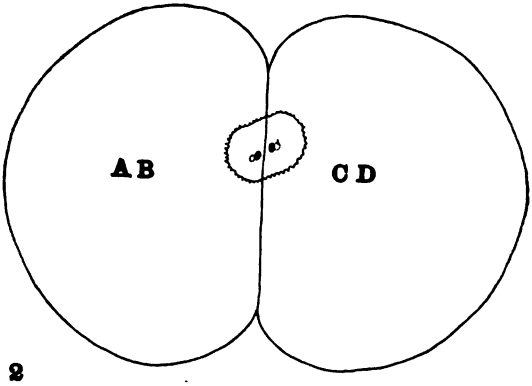
Fig. 34.—A stage in which about two-thirds of the embryo is covered by the shell.

Fig. 35.—Stage showing head vesicle (*H.V.*), larval heart (*L.H.*), mantle (*M.*), columellar muscle (*Cm.M.*), external kidneys (*Ex.K.*) and tentacles, (*T.*) in addition to the other organ primordia named in the description of figs. 28–30.

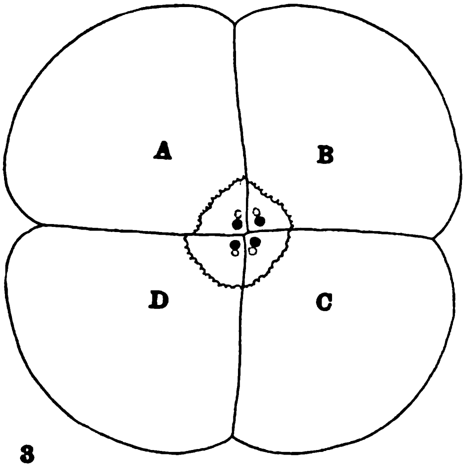
Fig. 36.—Later embryo, showing the formation of the mantle cavity. gill, velar lobes and the twisting of the nerve loop The primordia of all important organs are present at this stage.



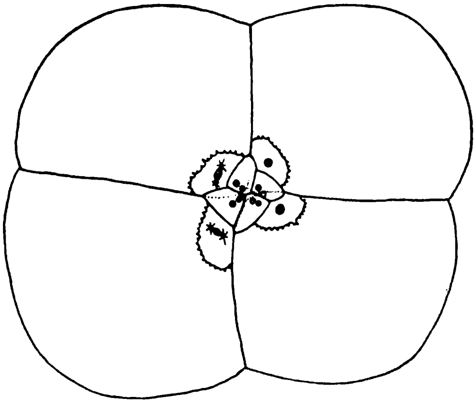
1



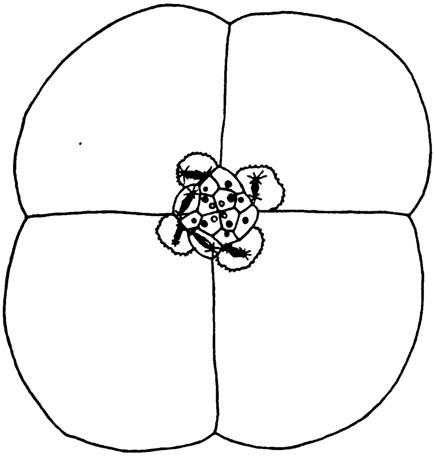
2



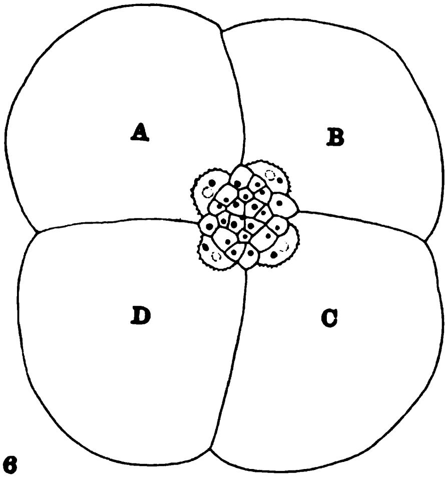
3



4

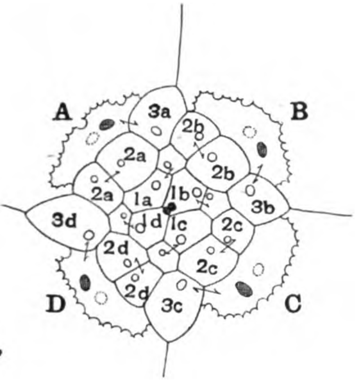


5

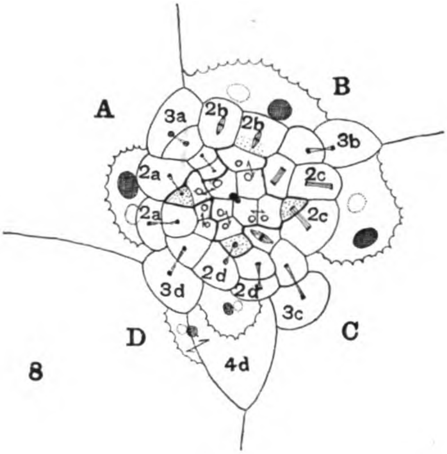


6

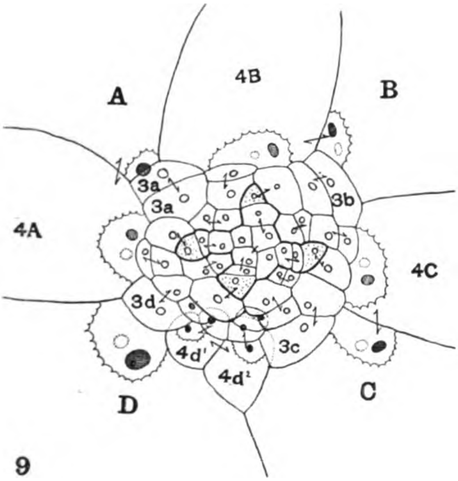
CONKLIN. EMBRYOLOGY OF FULGUR.



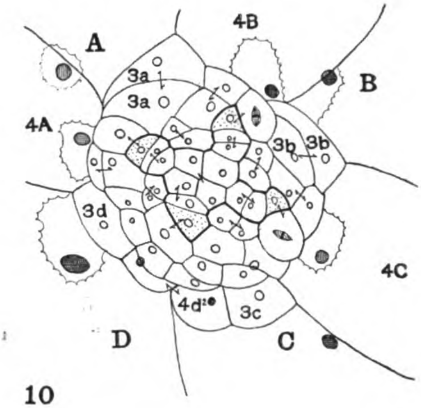
7



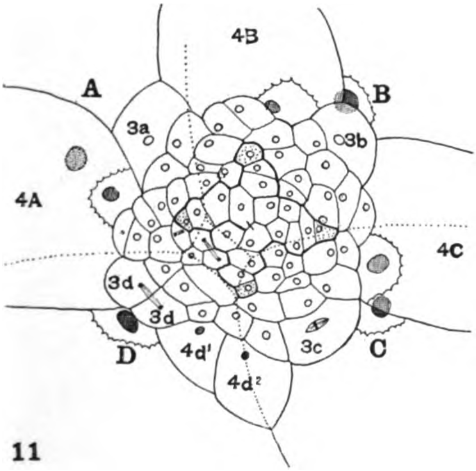
8



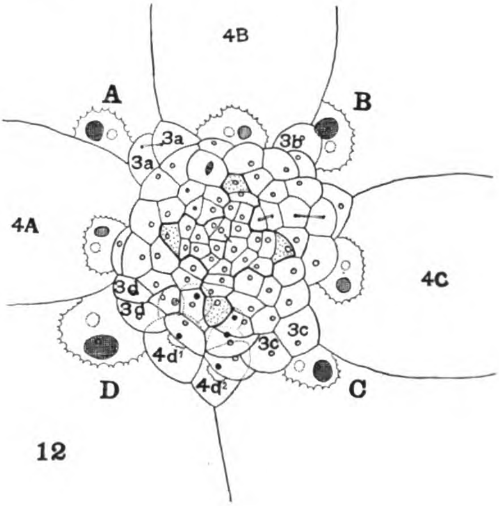
9



10

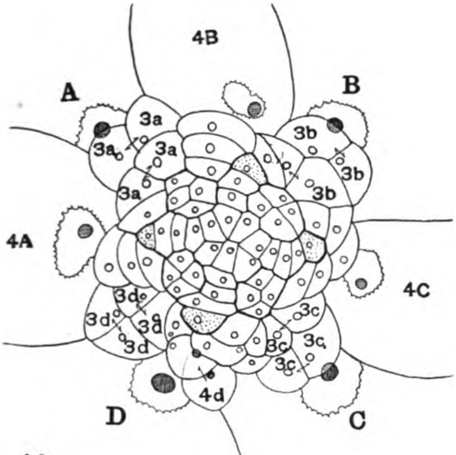


11

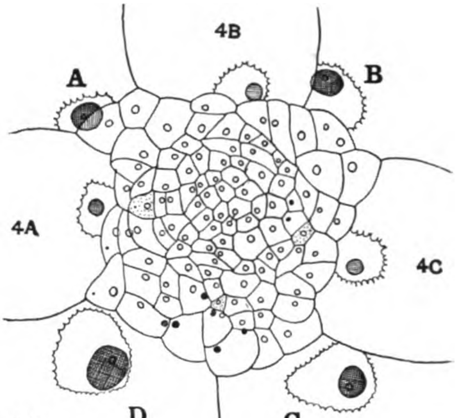


12

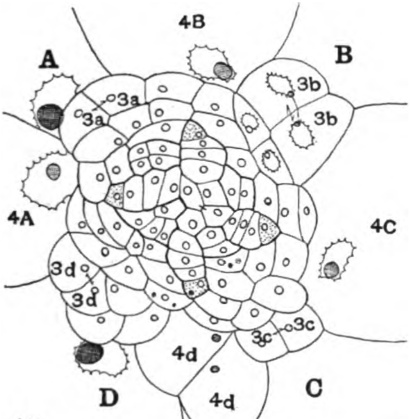
CONKLIN. EMBRYOLOGY OF FULGUR.



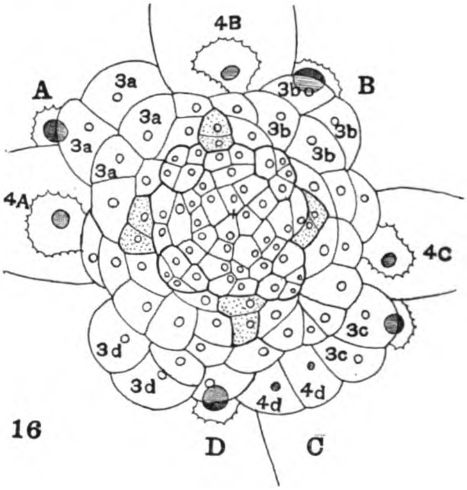
13



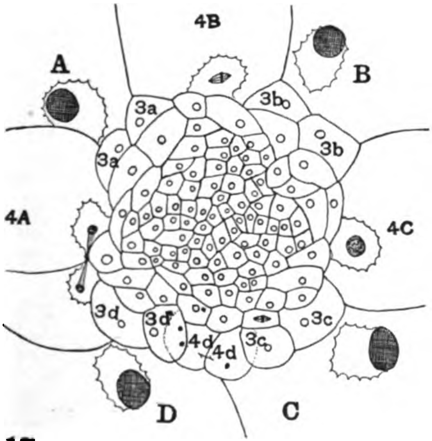
14



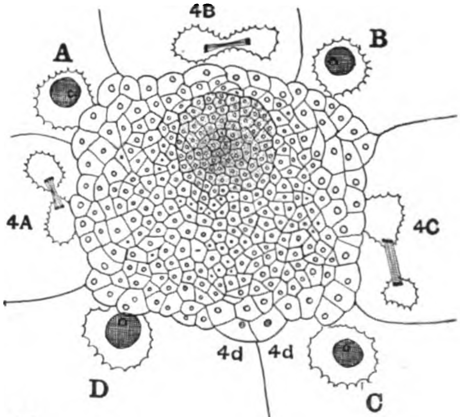
15



16

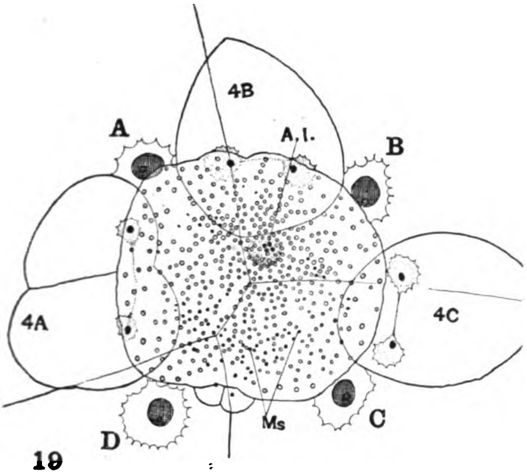


17

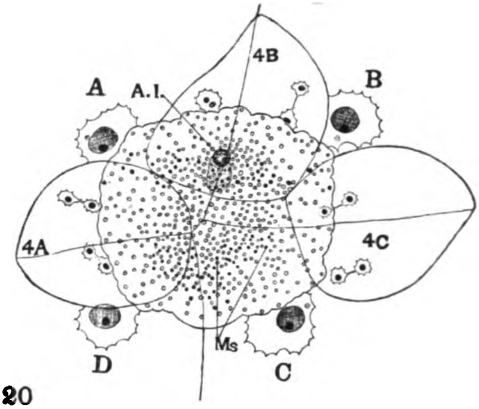


18

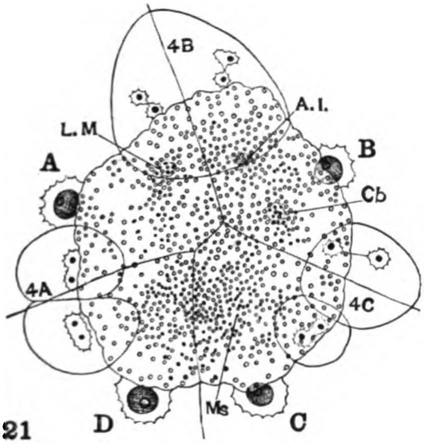
CONKLIN. EMBRYOLOGY OF FULGUR.



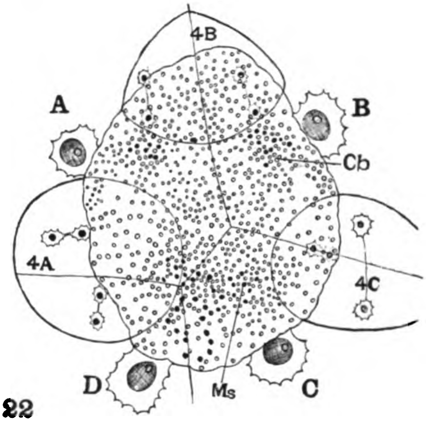
19



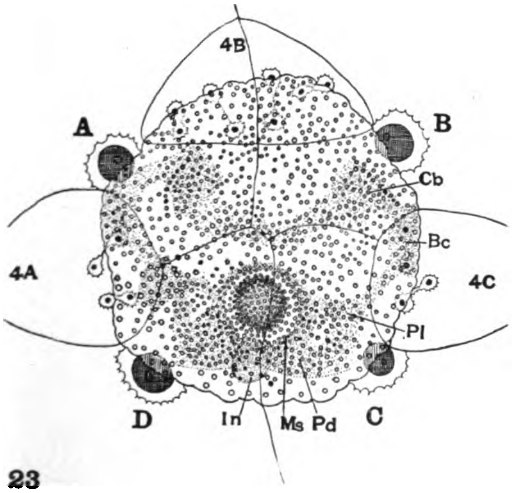
20



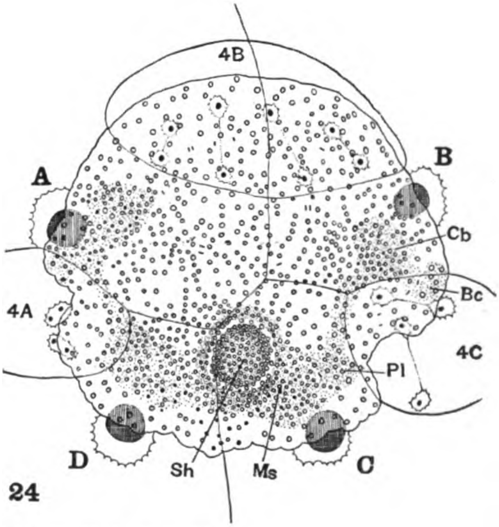
21



22

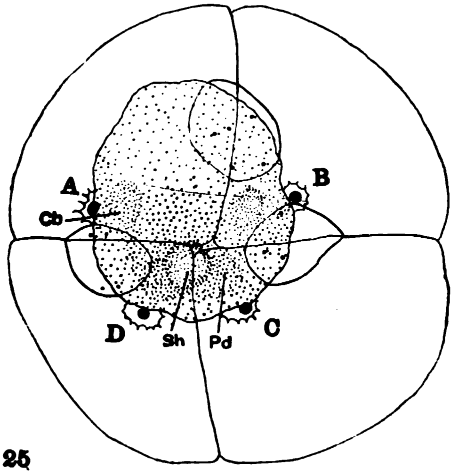


23

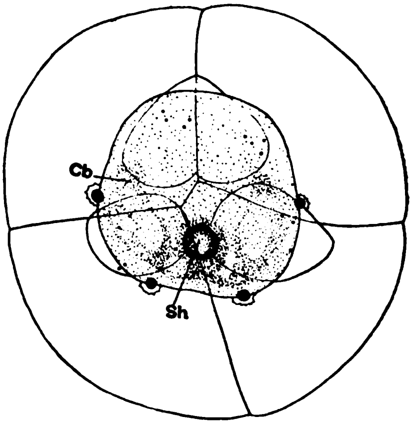


24

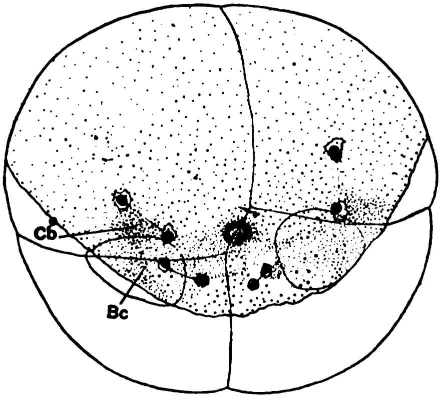
CONKLIN. EMBRYOLOGY OF FULGUR.



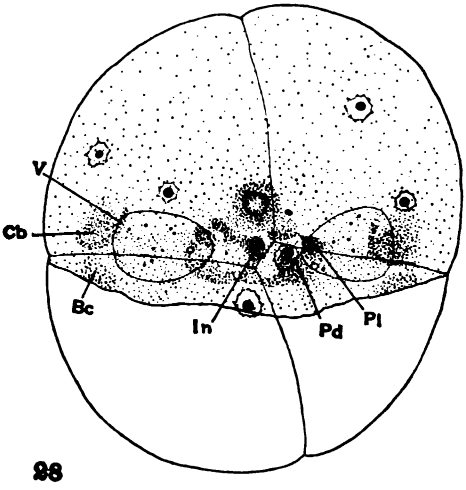
25



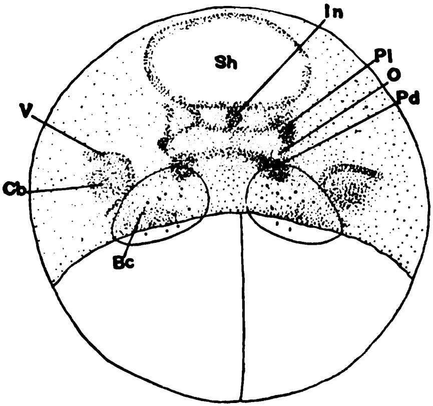
26



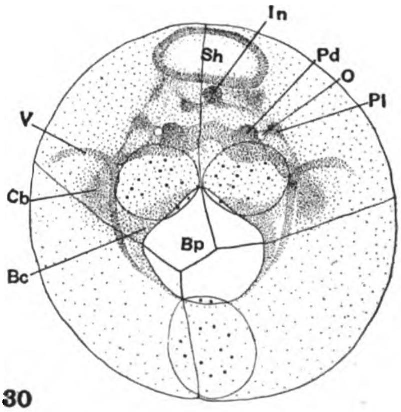
27



28

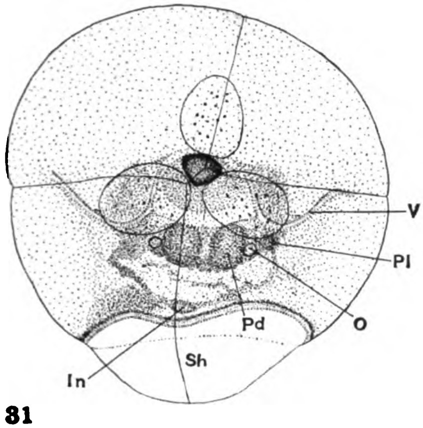


29

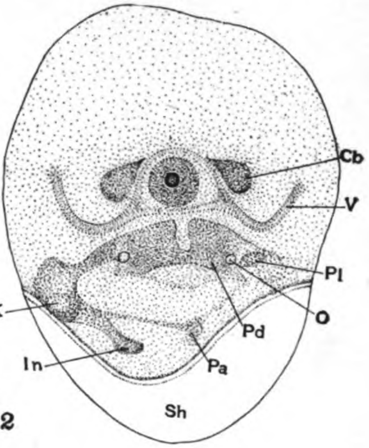


30

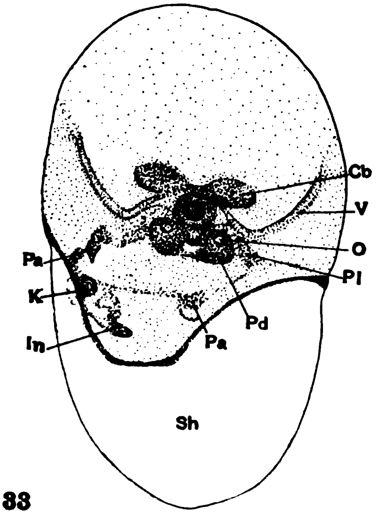
CONKLIN. EMBRYOLOGY OF FULGUR.



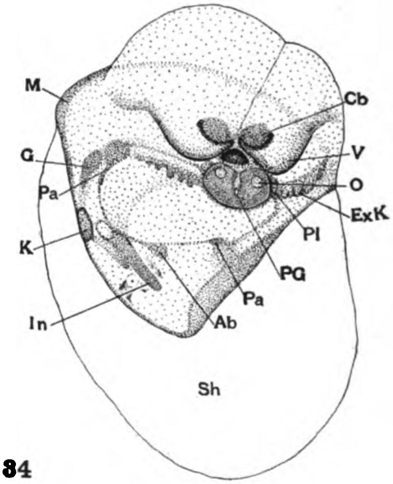
31



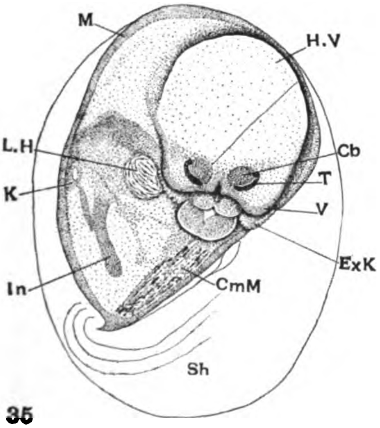
32



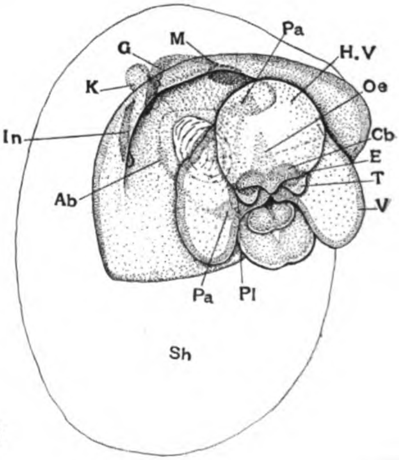
33



34



35



36

CONKLIN. EMBRYOLOGY OF FULGUR.

Notes on the True Neuroptera.

I.—Brachynemurus Curriei, a New Ant Lion from Texas.

By J. F. McClendon.

Brachynemurus curriei sp. nov.

Male.—Length 34 mm. Expanse of wings 34 mm. Mottled yellow and fuscous. Occiput yellow at sides, fuscous in the middle. Vertex fuscous with a narrow yellow stripe from eye to eye just behind the antennæ. The antennæ would reach back to the bases of the forewings; they are brown, with yellow rings marking the articulations; basal joint yellow; basal and second joints swollen. Front yellow, fuscous around the bases of the antennæ. Mandibles yellow, tipped with fuscous. Palpi yellow, tipped with fuscous. Pronotum a little longer than broad, tapering anteriorly, fuscous with a median longitudinal yellow band; a few long hairs. Mesothorax mottled yellow and fuscous; a few short hairs. Legs yellow with circular fuscous bands. Tibial spurs reach past three joints of the tarsus. Claws fuscous. Wing veins fuscous, interrupted with yellow. Pterostigma pale. In forewings a fuscous spot two-thirds the way between pterostigma and posterior margin. Abdomen fuscous, hairy. Genital appendages about as long as diameter of abdomen.

Female.—Length, 28 mm Expanse of wings, 40 mm. Stouter than male. Genital appendages shorter than diameter of abdomen. Other characters as in male.

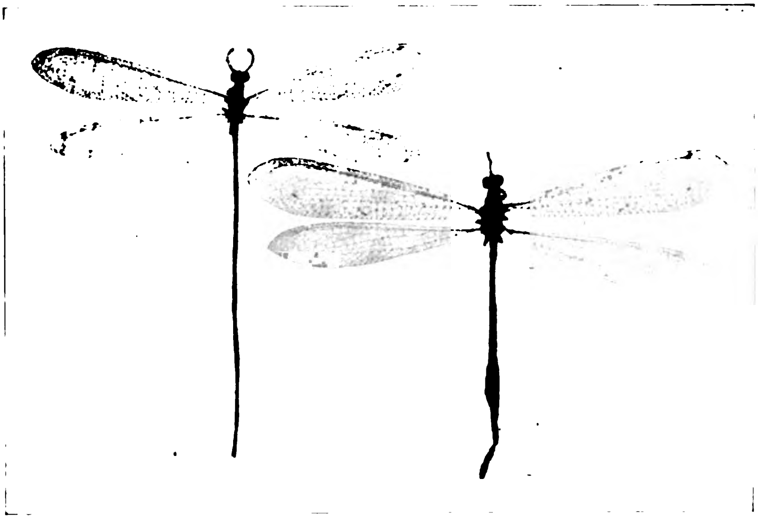


Fig. 1.—*Brachynemurus curriei* n. sp. Male to left and female to right, about 1⅓ natural size.

Type No. 9725 U. S. National Museum collected by Mr. H. S. Barber, at Brownsville, Texas. Cotypes: two males from Esperanzo, May 20, 1904, and Los Barregos, June 6, 1904, both in vicinity of Brownsville; six females from Brownsville and vicinity, all taken by Mr. Barber.

Notes on the True Neuroptera.

J. F. McCLENDON.

2.—*On Venation in Neuroptera.*

The wings of Neuroptera have been variously treated by systematists, and a few species have been considered by Comstock and Needham in their excellent series of articles in the 32d and 33d volumes of the American Naturalist. In 1901 I commenced to collect material for a developmental and comparative study of the venation of the group, but on account of pressure of other duties, failed to get many pupae, and had to content myself with studying the tracheation of the fully formed wings by bleaching them for days in chlorine water.

In distinguishing the veins, I have used the nomenclature of Redtenbacher as applied by Comstock and Needham.

SIALINA.

The wings of *Sialina* are broad and seemingly irregularly reticulated, but if we leave out of consideration the cross veins, the venation can easily be reduced to the hypothetical type of Comstock and Needham. The anal space of the hind wings is thin and folded when at rest.

SIALIDÆ.

In *Corydalis texana*, Fig. 1, the sub-costa and radius run parallel nearly to the tip of the wing, where they fuse. The radial sector has become pectinate by fusion of r^4 and r^5, as shown by Comstock and Needham. R^2 has three side branches,

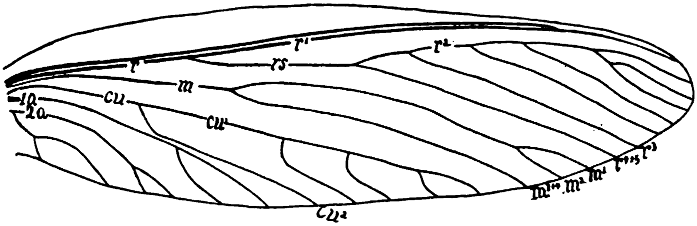


Fig. 1.—*Corydalis texana*. Fore wing. In this as well as in the succeeding figures the cross veins are omitted, save where they are represented by spaced lines.

one of which is divided into two. M^3 and m^4 are fused. Cu^1 has three side branches. The first, second, and third anal veins each branch once.

In *Sialis infumata*, Fig. 2, the anterior wing is modified by the fusion of cu^1 and m for some distance, and associated with this, the radius assumes a bowed form. M^1 and m^2 are fused. In the hind wing the bases of the median and cubitus are fused. M^1 and m^2 are fused. M^3 and m^4 are fused.

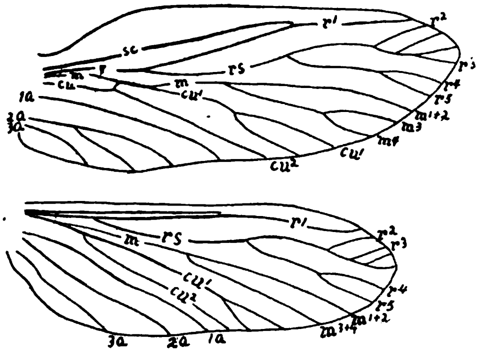


Fig. 2.—*Sialis infumata.*

RAPHIDIDÆ.

In *Raphidia oblita*, Figs. 3 and 4, the sub-costa reaches the margin of the wing before the pterostigma. In the anterior wing the radius, media, and cu^1 are fused for a short distance. In the posterior wing the bases of the radius and media are fused for some distance and cu^2 fuses with the first anal through the middle third of its course.

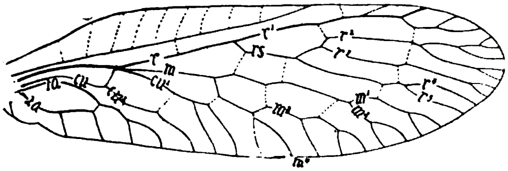


Fig. 3.—*Raphidia oblita.* Fore wing.

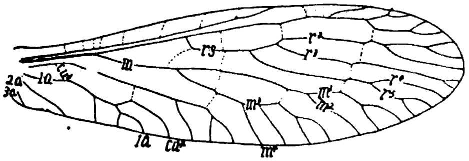


Fig. 4.—*Raphidia oblita.* Hind wing.

HEMEROBINA (MEGALOPTERA).

MYRMELEONIDÆ.

In the *Myrmeleonidæ* we have typical venation save that many accessory veins have been added distally and the branching of the median has been almost or quite completely suppressed.

In *Ululodes hyalina*, Fig. 5, the sub-costa and radius run parallel to the pterostigma, where they unite. The radial sector is branched dichotomously, though it is functionally pectinate, R^4 being united to R^{2+3} by a strong cross vein. The media is unbranched. R^2, r^5, Cu^1 and the anal furnish numerous veinlets by pectinate branching.

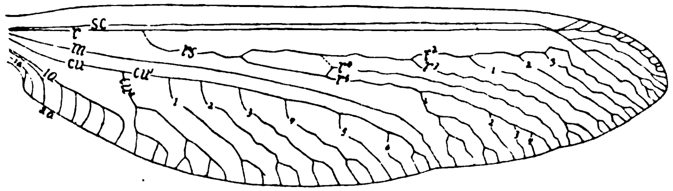


Fig. 5.—*Ululodes* (Ulula) *hyalina*. Fore wing.

In *Myrmeleon rusticus*, Fig. 6, the number of accessory veins is very much increased, Cu^1 having thirteen branches, each of which subdivides once.

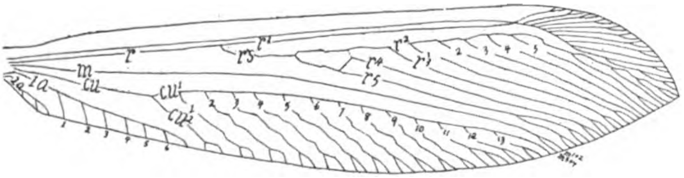


Fig. 6.—*Myrmeleon rusticus*. Fore wing.

HEMEROBIDÆ.

In the *Hemerobidæ* the radial sector has become pectinate by splitting of R^{4+5} as shown by Comstock and Needham for *Hemerobius*.

In the fore wing of *Micromus posticus*, Fig. 7, the radial sector is suppressed and the radius has become pectinate. Whether this has been caused by fission or coalescence, I have not enough material

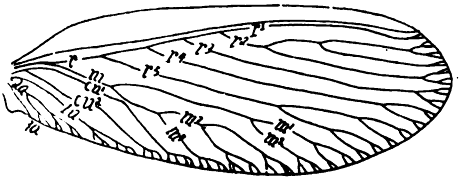


Fig. 7.—*Micromus posticus*. Fore wing.

to decide, but when we compare it with the hind wing, where the radial sector is distinct, we see the magnitude of the change. The cross-vein connecting r^1 with r^4 in the hind wing, Fig. 8, suggests that the change has been brought about by shifting of the trachea from longitudinal to cross veins—many tracheæ are found misplaced in the pupa, and this change might easily occur—the change would then be coalescence and not fission, as can be easily understood by studying the accompanying figures of the fore and hind wings. The sub-costa and r^1 run nearly parallel to the end of the wing.

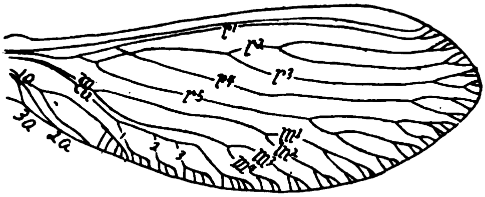


Fig. 8.—*Micromus posticus.* Hind wing.

In *Polystoechotes punctatus*, Fig. 9, R^2, by pectinate branching, gives rise to fourteen veinlets, each of which subdivides twice. Cu^1 has seven such branches. The sub-costa and r^1 fuse at the pterostigma and give rise to fifteen such veinlets.

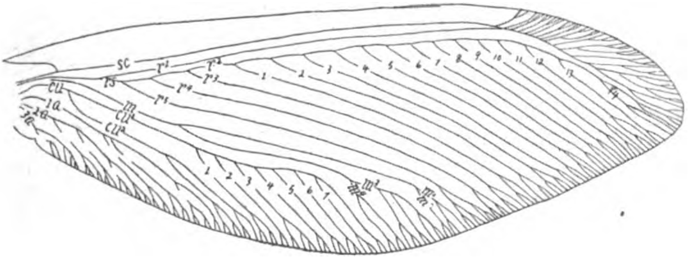


Fig. 9.—*Polystoechotes punctatus.* Fore wing.

MANTISPIDÆ.

In *Mantispa interrupta*, Figs. 10 and 11, the sub-costa runs through the middle of the pterostigma for the last third of its course, and disappears near its end. The base of

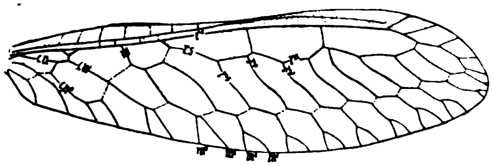


Fig. 10.—*Mantispa interrupta.* Fore wing.

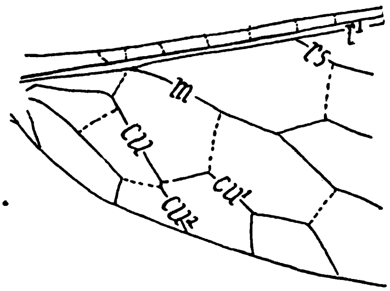


Fig. 11.—*Mantispa interrupta*. Part of hind wing.

the media fuses with that of the radius for some distance; and, in the anterior wing, Fig. 10, the media dips down and then fuses with the radius again thus forming a small triangular cell. After the media is finally free it divides into the typical number of branches.

CHRYSOPIDÆ.

In the *Chrysopidæ* we find a great modification by coalescence.

In the anterior wing of *Chrysopa plorabunda*, Fig. 12, the veins in the middle run zigzag and fuse at their angles, forming a reticular structure without the interposition of true cross veins. The so-called "cross vein" of the "third cubital cell" ($m^{3}+{}^{4}$) behaves in a very peculiar manner: $m^{3}+{}^{4}$ runs obliquely forward and coalesces for some distance with $m+{}^{2}$, and then separates from it again.

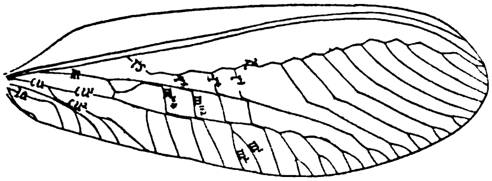


Fig. 12.—*Chrysopa plorabunda*. Fore wing. Where veins have coalesced I have represented them slightly separate for clearness of interpretation.

The hind wing, Fig. 13, resembles Myrmeleon, save that *rs* and *m* coalesce for some distance.

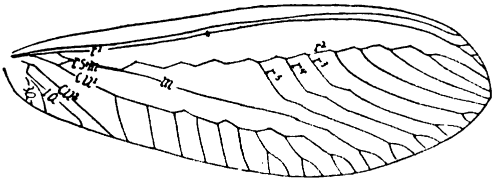


Fig 13.—*Chrysopa plorabunda*. Hind wing.

PANORPINA (MECAPTERA.)

PANORPIDÆ.

The venation of the *Panorpidæ* is quite typical, save for the fusion of veins near the base of the wing, due to narrowing of this region, and the development of a few accessory veins.

In *Panorpa confusa*, Fig. 14, r^2 branches once.

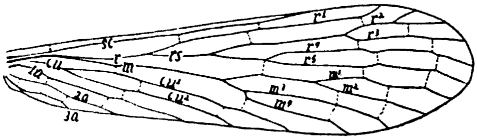


Fig. 14.—*Panorpa confusa*. Wing.

In *Bittacus strigosus*, Fig. 15, m and Cu^1 coalesce for some distance.

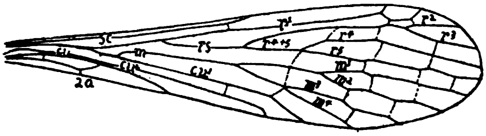


Fig. 15.—*Bittacus strigosus*. Wing.

Notes on the True Neuroptera.

By J. F. McCLENDON.

(Plate VIII)

3. *A Catalogue of Texas Neuroptera.*

Very few Neuroptera have been recorded from Texas, and I am writing the following list in the hope that it will encourage others to make a more thorough study of this interesting group in that region. Through the kindness of Dr. Samuel Henshaw I was allowed to examine the specimens in the Museum of Comparative Zoology, and Dr. L. O. Howard and Mr. R. P. Currie gave me every facility in looking over those in the National Museum. Mr. Nathan Banks, Asst. Entomologist, Dept. of Agriculture, let me study his collection, which contains more Texas material than that of any museum I have visited. Most Texas material is scanty and merely labeled "Texas," and in some cases the genus only could be identified. Where the collector's name was not known I have inserted the name of the owner or donor of the specimens. In many cases I refer to a monograph rather than the original description of a species, and have omitted the author's name where it is included in the name of the species.

SIALIDÆ.

SIALIS Latr. (*Sialis* sp.? Austin, McClendon).

CORYDALIS Latr.

cornuta Linn., Hagen, '61,* Columbus, E. A. Schwarz; Austin, McClendon.

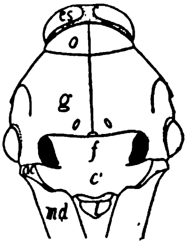


Fig. 1.—*Corydalis texana*, dorsal view of head and neck.

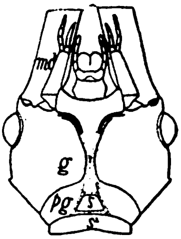


Fig. 2.—*Corydalis texana*, ventral view of head and neck.

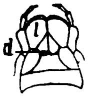


Fig. 3.—*Corydalis texana*, labium and hypopharynx of larva.

---

* Synopsis of Neuroptera of North America, by Hermann Hagen, Smithsonian Institute.

cognata Hag., '61,* Pecos River, Hagen.

crassicornis McLach., '67, Jour. Linn. Soc. Zool., v. 9, p. 233, San Antonio, A. Agassiz.

immabilis McLach., '67, Jour. Linn. Soc. Zool., v. 9, p. 235, Waco, Belfrage.

texana Banks, Jour. N. Y. Ent. Soc., v. 11, p. 239, Laredo, McClendon.

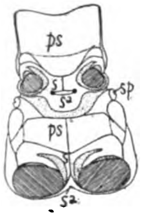


Fig. 4.—*Corydalis texana*, ventral view of thorax.

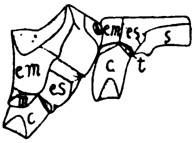


Fig. 5.—*Corydalis texana*, lateral view of thorax.

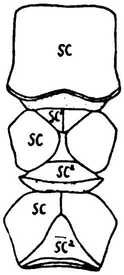


Fig. 30.—*Corydalis texana*, tergites of the thorax.

RAPHIDIDÆ.

RAPHIDIA Linn.

oculata Banks, '05, Trans. Am. Ent. Soc., v. 32, p. 4, Austin, McClendon.

MANTISPIDÆ.

MANTISPA Ill.

brunnea Say, Hagen, '61, Dallas, Riley; Victoria, Hinds, Morrill; San Antonio, Marlatt.

interrupta Say, Hagen, '61, Victoria, Caudell; Brownsville, E. A. Schwarz, H. Barber; San Antonio, Banks; Austin, McClendon.

CONIOPTERYGIDÆ.

CONIOPTERYX Halid. (*Coniopteryx* n sp., Brownsville, Barber).

CHRYSOPIDÆ.

LEUCOCHRYSA McLach.

antennata Banks, '05, Trans. Am. Ent. Soc., v. 32, p. 5, Brownsville, H. Barber.

americana Banks, '97, Proc. Ent. Soc. Wash., v. 4, p. 175, Austin, McClendon.

CHRYSOPA Leach.

oculata Say, Hagen, '61, Austin.

rufilabris Burm., Hagen, '61, Columbus, Schwarz; Victoria, Schwarz; Austin, McClendon.

quadripunctata Burm., Hagen, '61, Austin, McClendon.

sulphurea Fitch, Hagen, '61, Austin, McClendon.

plorabunda Fitch, Hagen, '61, Greenville, H. Barber; Austin, McClendon.

externa Hagen, '61, Greenville, H. Barber; Austin, McClendon.

punctinervis McLach., '69, Ent. Month Mag., '69, p. 24, Texas, McLach.

bimaculata McClendon, '01, Psyche, v. 9, p. 215, Brownesville, H. Barber; Austin, Banks; San Antonio, Banks; Laredo, McClendon.

HEMEROBIDÆ.

HEMEROBIUS Linn.

castaneæ Fitch, Hagen, '61, Texas, Belfrage.

perparvus McLach., '69, Ent. Month. Mag., '69, p. 21, Texas, McLachlan.

MICROMUS Rambur.

angustus Hagen, '86, Proc. Bost. Soc. Nat. Hist., '86, p. 287, Texas, Belfrage.

insipidus Hagen, '61, Columbus, Schwarz.

posticus Walk., B. M. Cat., Neur., p. 283, Victoria, Schwarz; Austin, McClendon.

CLIMACIA McLachlan.

areolis Hagen, '61, Columbus, Schwarz; Texas, Belfrage.

LOMAYIA Banks.

texana Banks, '97, Trans. Am. Ent. Soc., v. 4, p. 24, Brownsville, H. Banks; Central Texas, Banks.

MYRMELEONIDÆ.

(MYRMELEONINÆ.)

ACANTHACLISIS Rambur.

texana Hagen, '87-8, Canad. Ent., p. 197, Texas, Hagen.

DENDROLEON Hagen.

obsoletus Say, Hagen, '61, Blanco Co., Am. Ent. Soc.

PSAMMOLEON Banks.

ingeniosus Walk., Hagen, '61, Columbus, Riley; Brownsville and Alice, Barber; Blanco Co., Am. Ent. Soc.; Austin, McClendon.

BRACHYNEMURUS Hagen.

carrizonus Hag., '87-8, Canad. Ent., p. 93, Blanco Co., Am. Ent. Soc.; Brownsville, H. Barber; San Diego, Schwarz; College Station, Banks; Carrizo Springs, Hagen; Austin, McClendon.

abdominalis Say, Hagen, '61, Brazos Co., Banks; Victoria, W. E. Hinds; Alice, H. Barber; Blanco Co., Am. Ent. Soc.; Dallas, Boll; Carrizo Springs, Hagen; Austin, McClendon.

longicaudus Burm., Hagen, '61, Texas, Riley.

peregrinus Hag., '61, Matamoros, Hagen.

sackeni Hag., '87-8, Round Mountain, Am. Ent. Soc.; Dallas, Boll.

irregularis Currie, '06, Proc. Ent. Soc., Wash.; Columbus, Schwarz.

hubbardi Currie, '98, Canad. Ent., v. 30, p. 93,=*cockerelli* Banks; Carrizo springs, Dr. A. Wadgymar; Columbus, Am. Ent. Soc.

texanus Banks, '03, Jour. N. Y. Ent. Soc., '03, p. 240, Amarillo, Cockerell; Laredo, McClendon.

dorsalis Banks, '63, Jour. N. Y. Ent. Soc., '03, p. 240, Laredo.

curriei McClendon, '06, ENT. NEWS, v. 17, p. 93, Brownsville, Esperanzo, Los Barregos, Barber.

MYRMELEON Linn.

immaculatus DeGeer, Hagen, '61, Texas, Hagen.

rusticus Hagen, '61, Brownsville, H. Barber; Victoria, Caudell, J. Mitchell, Schwarz; Columbus, San Diego, Schwarz; Pecos River, Hagen; Matamoros, Hagen; Austin and Laredo, McClendon.

texanus Banks, '00, ENT. NEWS, v. 11, p. 596, Columbus, Schwarz; Galveston, McClendon.

NEUROPTYNX n. gen. (= *Ptynx* Lefeb., preoccupied).

juvenilis McLach., '71, Jour. Linn. Soc. Zool., Dallas Boll.; Austin McClendon.

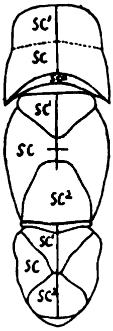


Fig. 31.—*Myrmeleon rusticus*, tergites of the thorax.

ULULODES Currie (*Ulula* Ramb.)

hyalina Latr. Hagen, '61, Dallas, H. Barber, Boll.; Austin, A. W. Morrill, McClendon; Bosque Co., Belfrage; Columbus, Schwarz; Waco, Galveston and Laredo, McClendon; Brownsville, H. Barber.

PANORPIDÆ.

BITTACUS Latr.

occidentis Walk., Hagen, '61, Texas, Banks.

PANORPA Linn.

nuptialis Gerst., '63, Stet. Ent. Zeit. '63, p. 24, Jonesville, Mally; College Station, Banks; Victoria, Calvert, A. W. Morrill; Dallas, Boll; Austin, McClendon, Norman.

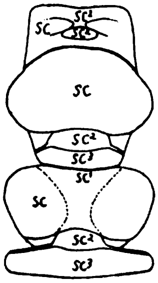


Fig. 32.—*Panorpa nuptialis*, tergites of the thorax.

EXPLANATION OF FIGURES.

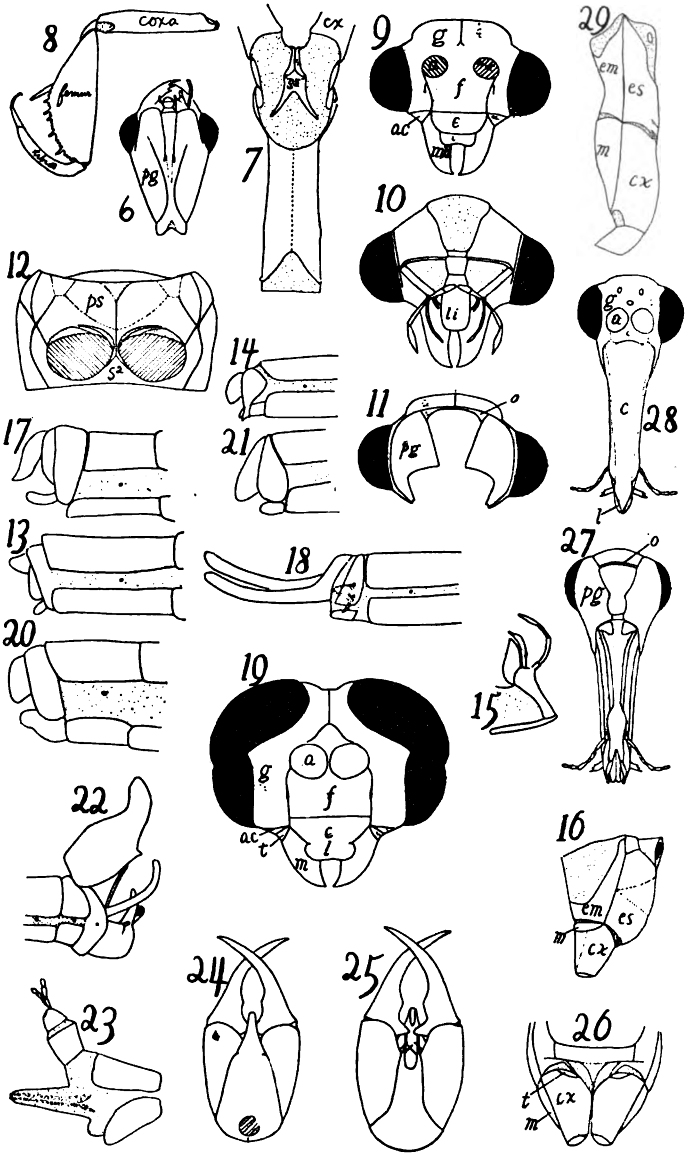
The accompanying figures are intended to illustrate some points in the external anatomy. Where appendages are cut off, the cut surface is usually shaded with parallel lines. Membranous parts are shaded lightly with dots.

ABBREVIATIONS.

a.	Antenna.
ac.	Antecoxal piece.
c.	Clypeus.
c^1.	First Clypeus.
c^2.	Second Clypeus.
cx.	Coxa.
em.	Epimeron.
es.	Episternum.
f.	Front.
g.	Gena.
gu.	Gula.
l.	Labrum.
li	Labium.
m.	Meron.
md.	Mandible.
mx.	Maxilla.
o.	Occiput.
pg.	Postgena.
ps.	Presternum.
s.	Sternum.
s^2	Sternellum.
sc^1.	Prescutum.
sc.	Scutum.
sc^2	Scutellum.
sc^3.	Postscutellum.
sp.	Spiracle.
t.	Trochantine.

EXPLANATION OF PLATE VIII.

Fig. 6. *Raphidia oblita*, ventral view of head.
" 7. *Mantispa brunnea*, ventral view of prothorax.
" 8. " " anterior leg.
" 9. *Myrmeleon rusticus* dorsal view of head.
" 10. " " ventral " " "
" 11. " " posterior view of head.
" 12. " " ventral " " mesothorax.
" 13. " " caudal appendages of female.
" 14. " " " " " male.
" 15. " " maxilla.
" 16. " " lateral view of mesothorax.
" 17. *Brachynemurus carrizonus*, caudal appendages of female.
" 18. " " " " " male.
Inner parts shaded.
" 19. *Ululodes hyalina*, head.
" 20. " " caudal appendages of female.
" 21. " " " " " male.
" 22. *Bittacus strigosus*, " " " "
" 23. *Panorpa nuptialis* " " " female; the abdomen is pressed so as to extrude the ovipositor, which is semi-transparent.
" 24. " " caudal appendages of male, dorsal view.
" 25. " " " " " " ventral view.
" 26. " " ventral view of mesothorax.
" 27. " " anterior view of head.
" 28. " " posterior " " "
" 29. " " lateral view of mesothorax.



THE TRUE NEUROPTERA McCLENDON.

[Reprinted from BIOLOGICAL BULLETIN, Vol. XII., No. 1, December, 1906.]

ON THE DEVELOPMENT OF PARASITIC COPEPODS.[1]

PART I.

J. F. McCLENDON.

CONTENTS.

I. Introduction 37
II. Historical 39
III. Anatomy of the reproductive organs 39
IV. Oögenesis and maturation 42
  1. Oögenesis 42
    A. The dichelestid 42
    B. *Læmargus muricatus* 43
    C. *Pandarus sinuatus* 44
  2. Maturation 44
    A. *Pandarus sinuatus* 44
    B. *Læmargus muricatus* 45
    C. The dichelestid 45
V. Spermatogenesis 47
VI. Polarity of the egg 49
VII. Summary 50
VIII. Bibliography (see part II. of this paper) 76
IX. Explanation of plate 50

The work herein presented was begun at Wood's Hole, 1904, with a view of determining the effects of pressure on the eggs of parasitic copepods. Owing to technical difficulties, three summers have passed with few experimental results, and in consequence the present paper is chiefly of a morphological nature.

I obtained parasitic copepods from fish caught in traps of the Marine Biological Laboratory and the U. S. Fish Commission, at Woods Hole. It was usually necessary to go to the trap to get them fresh, or in the case of some Caligidæ to catch them before they left the fish.

Most of the work was done on *Pandarus sinuatus* Verrill, *Læmargus muricatus* Kröyer (collected by Dr. Conklin), and a new species (text Fig. 3) of the family Dichelesteidæ and of a new genus near *Kröyeria* (according to Prof. Charles B. Wilson) which I will call throughout this paper the dichelestid. The eggs of *Pandarus sinuatus* are the most difficult to handle, being very flat and thin and pigmented, but I used them because this is the most common species at Woods Hole. I used the eggs of the following for comparison (arranged according to the classification of Claus):

---

[1] Thesis accepted by the Faculty of the Department of Philosophy of the University of Pennsylvania toward the degree of Doctor of Philosophy.

Caligidæ:
- *Caligus bonito* Wilson (and several undetermined species of *Caligus*).
- *Caligus rapax* Milne Edwards.
- *Lepioptherius edwardsi* Wilson.
- *Perissopus communis* Rathbun.
- *Nesippus alatus* Wilson.
- *Cecrops latreillii* Leach.
- *Læmargus*[1] *muricatus* Kröyer (and an undetermined species of *Læmargus*).
- *Philorthagoriscus serratus* Kröyer.
- *Pandarus sinuatus* Verrill.

Dichelesthiidæ:
- *Anthosoma crassum* Abilguard.
- *Kröyeria ?*
- *Eudactylina nigra* Wilson and *E.* sp.?

Lernæidæ:
- *Penella.*

Chondracanthidæ:
- *Chondracanthus.*
- *Sphyrion ?*

For the determination of species I am indebted to Prof. Charles Branch Wilson, of Massachusetts State Normal School, Westfield, Mass., who has been very kind in his interest in my work.

After my work was nearly finished I received the material collected at Woods Hole in 1899 by Prof. Edw. Rynearson, of Pittsburgh, with a view of working on this same subject, and which he kindly turned over to me. I wish to express my thanks for this abundance of material, which allowed me to confirm many points on which my material was scanty and to add some new ones.

---

[1] The word *Læmargus* was applied to a copepod and a shark in the year 1837 and as yet it is disputed which should claim priority.

I am indebted to Dr. Conklin for constant guidance and assistance besides the general direction of my work, and to Mr. Kribs for the use of Zeiss apochromatic lenses.

Dr. Formad and Dr. Fischelis rendered me invaluable service in translating Russian.

I am under obligations to the Carnegie Institute for a table in the Marine Biological Laboratory, Wood's Hole, 1904, and to the U. S. Fish Commission for a table in the U. S. F. C. Laboratory, Wood's Hole, Mass., 1905–6.

The material was fixed in Flemming's fluid, picro-acetic, or corrosive-sublimate-acetic. The first gave the best fixation but the second was the most convenient. Heidenhain's iron hæmatoxylin followed by various counter-stains, and Hermann's safranin-gentian violet were used most frequently.

II. Historical.

The ground covered in this paper was almost entirely untouched by earlier workers as regards the particular genera treated, and I will consider here only some papers on related forms.

The free living copepods have long been favorite objects for study. Gruber ('79) described their reproductive organs. Weismann and Ischikawa ('88 *a*, *b*) and Ischikawa ('92) used them in studying the question of reduction in number of chromosomes. Haecker ('91, '92 *a*, *b*, '94 *a*, *b*, '95 *a*, *b*, '97, '02) found them favorable objects for study of oögenesis, maturation, reduction, and unsymmetrical mitoses. Later, Rückert ('94, '95 *a*, *b*, *c*) studied oögenesis, maturation, and reduction in these.

Of the parasitic and half parasitic copepods, Heider ('79) notes the spermatogenesis of *Lernanthropus*, and Giesbrecht ('82) the oögenesis of the Notodelphidæ.

Charles Branch Wilson in his monograph on the Caligidæ ('05) describes the anatomy of the reproductive organs and the life history.

III. Anatomy of the Reproductive Organs.

In *Pandarus*, *Caligus* and allied forms the two ovaries lie in the head (Fig. 1) and the two oviducts run backwards to the genital segment, where they are much convoluted and increase greatly in diameter and each communicates by an opening, the os uteri, with an egg string that trails behind the animal. The ovary is formed of a much convoluted cord of cells in a single linear series, small at the oögonial end and gradually increasing in diameter until it passes into the oviduct. As these cells (oöcytes) grow in the ovary they become much compressed in the direction of the long axis of the cord and the cell boundaries almost completely disappear. Passing into the oviduct, the oöcytes continue to grow and soon their boundaries reappear, and they become more flattened as they grow larger. There are at least two broods in the oviduct at once, caused by the periodic activity of the ovary. The younger brood extends some distance into the genital segment and is sharply marked off from the older brood, which occupies the last coils of the oviduct and consists of oöcytes that have nearly or quite completed their growth. As the oöcytes pass out through the os uteri they are fertilized from the seminal receptacles (Fig. 2, *sr*) and surrounded with secretion from the cement gland (*c.g.*) which forms the wall of the egg string. The eggs are distorted when passing through the thorax, but in most cases regain their symmetrical form. Embryos in the egg strings have their heads turned latero-ventrally and their ventral surfaces anteriorly, in relation to the mother. Occasionally one finds an embryo reversed, or partially rotated.

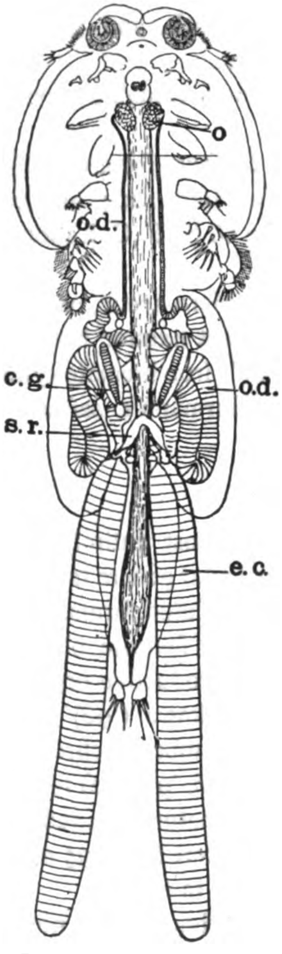


FIG. 1. Female reproductive organs of Caligus bonito. (Drawn by Emerton from Wilson's *Caligidæ.*) *c. g.*, cement gland; *e. c.*, external egg cases; *o*, ovary; *o. d.*, oviduct; *s. r.*, semen receptacle.

In the male the same general arrangement of reproductive organs exists, save that the seminal receptacles are absent and the distal ends of the vasa deferentia are enlarged and become the spermatophore receptacles. The cement glands secrete the material for the walls of the spermatophores.

In the dichelestid (FIG. 3) the genital segment is very much elongated and the oviducts are not convoluted. The ovaries have been carried backward until they lie in the anterior end of the genital segment and the oviducts pass forward to the anterior end of the thorax, then backward to the attachment of the egg strings. In other respects the description given for *Caligus* will hold for this species. The older brood of oöcytes occupies the posterior two-thirds of the oviduct. When one brood passes into the egg string the distal eggs of the remaining brood have nothing to press against and tend to round up and become distorted. The thickness of the oviduct at its posterior end varies somewhat, depending on the number of eggs produced in one brood, and the size of the eggs. When fewer, the eggs are thicker, and when more numerous, thinner. The egg string is more slender than the posterior portion of the oviduct, and the contained eggs are, therefore, thicker.

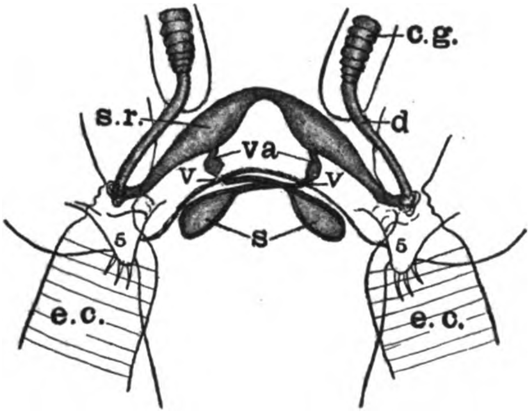
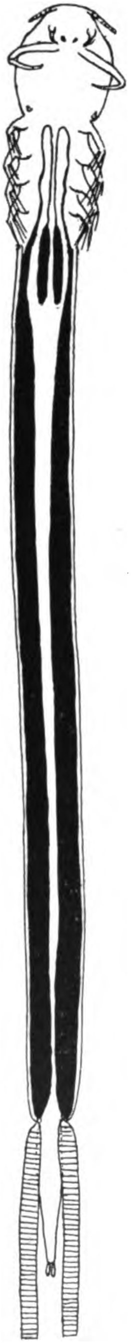


FIG. 2. Semen receptacles and vagina of a female *Lepeophtheirus*. (Partly after Claus.) *c. g.*, cement glands; *d.*, cement gland duct; *e. c.*, egg cases; *s.*, spermatophores; *s. r.*, spermaries; *v.*, vulva; *va.*, vagina; 5, fifth legs. (From Wilson's *Caligidæ.*)



In *Læmargus* the egg strings are packed in loops under broad lamellar coverings.

IV. Oögenesis and Maturation.

1. *Oögenesis.*

A. The Dichelestid. — The oögonia (Fig. 1) are very small isodiametrical cells. The nucleus is spherical and contains chromatin granules in a peripheral linin reticulum. Minute nucleoli are also embedded in this reticulum. The cytoplasm stains deeply. I have found but few oögonial mitoses in this species although I have sectioned over a hundred females; the number of chromosomes is the same as in the primary germ cells of the embryo (16) and *twice* the reduced number (8).

The primary oöcyte is readily distinguished from the oögonium by its nucleus being about half the size of the latter (when first formed). The earliest stage I have is the late telophase, in which the chromosomes are drawn together as though at the pole of the spindle, yet the whole mass is surrounded by a nuclear membrane (Fig. 2). The nuclear membrane is therefore formed of the fused linin sheaths of the chromosomes (or from cytoplasm ?). This mass is placed excentrically in the nucleus and the individual chromosomes are so pressed together or fused that only their ends sticking out can be distinguished separately, so that it resembles a "synapsis." The cytoplasm stains deeply. The oöcytes are arranged in single linear series as stated above and are pressed and flattened one against the other; the nuclei are close to the free surfaces of the cells, and that edge of an oöcyte containing the nucleus is thicker than the opposite edge. Soon the chromosomes swell and the chromatin becomes

FIG. 3. The *Dichelestid*, ventral view of ♀. The ovaries and oviducts are solid black. Portions of the egg strings are still attached.

dispersed as granules in a peripheral achromatic reticulum, as in the oōgonia (Fig. 3). Minute nucleoli appear in this reticulum. The boundaries between the oōcytes become so faint as to be no longer everywhere perceptible. At the same time the cytoplasm begins to increase in volume and the diameter of the egg cord to increase. This process continues until the egg cord passes into the oviduct, and for some time thereafter. When the oōcytes have traversed about a fifth of the oviduct, the cell boundaries reappear and the nucleus migrates to very near the center, there forming an enlargement in the oōcyte (Fig. 4). The reticulum and the three to four nucleoli have grown considerably with the growth of the nucleus. A few yolk spherules and fewer oil globules appear and grow to considerable size. For some time growth of the oōcyte consists almost solely in the addition of yolk spherules and oil globules and this continues until the yolk almost obliterates the cytoplasm and closely surrounds the nucleus. I have seen these spherules and oil globules separated from the protoplasm with a high speed centrifuge (kindness of Dr. Lyon), the former are heavier and the latter lighter than the protoplasm.

B. Læmargus muricatus. — The oōgenesis resembles that of the dichelestid. In the oōgonial divisions there appear to be sixteen chromosomes, but these are set so close together that I cannot be sure by actual count.

In the growth period the reserve materials, yolk and oil globules, are laid down in ways that show them to be of quite different consistency. The oil globules (dissolved out in sections) appear as minute points that grow in size but always retain a spherical shape. The yolk substances, at least some of them, are laid down as thin discs. A disc receives new substance on its flat surfaces only, and the layers of substances are alternately chromatic and achromatic in staining qualities (when much destained after certain stains). Some of these piles of discs become spherical, others oblong by addition of more discs. The constituent layers do not mix but remain separate until dissoved in the segmenting egg or developing embryo. The oil globules are relatively few in number and large in size. The yolk bodies vary greatly in size, none of them reaching the size of the oil globules.

C. Pandarus sinuatus. — The oöcyte of *Pandarus sinuatus* differs from that of the dichelestid in that a single nucleolus is formed, about equal in bulk to that of the several nucleoli of the latter species together. The oöcyte of the former is much more compressed (thinner) than that of the latter.

2. *Maturation.*

A. Pandarus sinuatus. — At the end of the growth period the nucleus becomes irregular in outline which gives it a shrunken appearance. The nucleolus becomes vacuolated and the nuclear sap intensely staining. The location of chromatin cannot be made out very well, but in thin sections chromatic threads can be seen radiating from the nucleolus. Soon the nuclear sap fades enough to show that the chromatic threads have split (Fig. 6). The nuclear wall is dissolved. Sometimes the vacuoles in the nucleolus increase very much in size and fuse into one. In preparations of the entire oöcyte the chromatic thread can be seen to be divided into eight double chromosomes (Fig. 5). By shortening of these double-rod-shaped chromosomes, ring-shaped chromosomes are formed (Fig. 7). A transverse constriction transforms the diad into a tetrad, that is a ring constricted at four equidistant points, the two opposite constrictions, representing the divisions between the original rods, being deeper than the other two. The spindle when first formed is longer than the shortest diameter of the egg. It is similar to that found in free living copepods, having no polar rays. The dense protoplasm at each end of the spindle (Figs. 7 and 9) may possibly be derived from archoplasm of preceding divisions, and it shows a striking resemblance to the pole plates of the dividing nucleus in Protozoa. The spindle is at first parallel to the flat surfaces of the egg and rotates to an almost vertical position (Fig. 8). The first polar body is very small and is extruded between the egg and its neighbor at about the center of the flat surface that is posterior (in relation to the mother) (Figs. 9 and 10). The second polar spindle is smaller than the first. It is at first parallel to the flat surfaces of the egg and rotates nearly to a perpendicular position under the first polar body (Figs. 9 and 10). I have not seen the second polar body being cut off and have distinguished only one polar body in later stages, which on account of its size I regard as the first polar body or both polar bodies fused (Fig. 49, *P*). It may be that the second polar body remains in the egg as in *Cyclops* and is not easily distinguishable, or more probably it is extruded immediately under the first polar body and the two being pressed together and becoming very flat, appear as one. The chromosomes in the first polar body do not swell and fuse to form a nucleus.

I have made no observations on the entrance of the sperm. Vide under Polarity of the Egg.

B. Læmargus muricatus (Fig. 18). — Maturation in this species is very similar to the process in *Pandarus*. The disintegration of the single large nucleolus shows many stages which appear to be peculiar to this species. A number of vacuoles (globules of achromatic fluid) appear in it, and these sometimes fuse into a single mass. The chromatic substance does not remain as a continuous peripheral layer but rounds up into four or more masses whose inner surfaces are hemispherical and outer surfaces form portions of the general surface of the nucleolus. In some cases there are two fluids (one colorless and the other staining very faintly) formed within the nucleolus. The colorless fluid forms a large sphere in the center and the faintly staining fluid occupies the periphery and contains spheres of the original chromatic substance of the nucleolus. The chromosomes do not go as far in metamorphosis as in *Pandarus*. In the equatorial plate the chromosomes appear as short double rods (Fig. 18), and if we regard the spireme formed of chromosomes joined end to end and cut into half the usual number of pieces the first maturation division is equational and the second reducing. On the other hand if chromosomes pair in the synapsis stage and lie parallel to form double rods, the first division is reducing and the second equational. The chromosomes cannot be distinguished all through the resting period, and whereas I am inclined toward the view that each rod of the double rod represents a chromosome, I have not sufficient direct evidence to be sure that this is the case.

C. The Dichelestid. — Toward the close of the growth period the nucleus undergoes an enormous change. The nucleoli become vacuolated and irregular in outline. The reticulum containing chromatin begins to break up and the nuclear sap stains intensely, probably due to the solution of chromatin. The nucleus becomes irregular in outline, probably due to pressure of yolk. From one to three spheres of protoplasm (Fig. 11) are found in some eggs near the wall of the oviduct, which appear to be abnormal structures. With the breaking up of the reticulum, the chromosomes are formed, but the manner in which this occurs is obscured by the intense staining of the nuclear sap. (This process shows better in *Pandarus.*) In order to see any structures in the nucleus it must be de-stained until it is very faint. The nuclear membrane disappears and the karyoplasm increases in volume and soon some of the chromosomes are distinctly double. The nucleoli disappear suddenly. The karyoplasm fades somewhat and becomes filled with alveoles, some of which are very large (Fig. 12). Each chromosome, of which there are eight (Fig. 13), is surrounded by a sphere of homogeneous protoplasm that stains slightly deeper than the surrounding cytoplasm. Each chromosome is a tetrad and often opens out into a ring constricted at four equidistant points. Two opposite constrictions are deeper than the other two and are to be regarded (from comparison with *Læmargus* and *Pandarus*) as the first divisions formed, and probably divisions between whole chromosomes. When seen on edge the tetrad usually appears dumb-bell shaped. There is some variation in the shape of the chromosomes in early prophases (Fig. 12) but in later stages they appear quite uniform in size and shape (Fig. 14). The spheres of homogeneous protoplasm surrounding the chromosomes fuse into one mass. A colorless area appears around each chromosome (Fig. 13) which makes it appear as though each chromosome was enclosed in a linin sac.

These linin "sacs" are each drawn out in the form of a spindle, and these spindles, lying parallel, form the first maturation spindle. Spindle fibers develop (Figs. 13 and 14) and some of them become attached to the chromosomes. The spindle is elongated, and at each pole the protoplasm stains more intensely resembling the pole plate in protozoön mitosis (Fig. 16). The paler protoplasm forms a sphere around each pole of the spindle, (Fig. 16). From these spheres radiate strands of protoplasm simulating astral rays, but not so dense. The spindle is formed parallel to the flat surfaces of the egg and then begins to rotate to a perpendicular position, at the same time shortening (Fig. 17). In this state it remains until fertilization, and further than this I have not followed it. The behavior of the linin sheath of the chromosome is very peculiar, and it seems to be more conspicuous than in any other instance I know of. The spindle, in general appearance, resembles the first cleavage spindle of *Cyclops* (Häcker).

V. SPERMATOGENESIS.

Læmargus muricatus. (*Preliminary Notice.*)

The spermatogonia are small cells with comparatively large spherical nuclei, and are arranged in single linear series. The nucleus contains chromatin granules in a peripherical linin reticulum. When preparing for mitosis these granules become arranged into looped rows, but I have not ascertained whether these rows form a continuous spireme or not. There is a single large nucleolus. In the last spermatogonic divisions there are 16 chromosomes (twice the number of the spermatocytic divisions). The preliminary spermatocytes are half the size of the spermatogonia when first formed, and are arranged in single linear series. Whether there is a single chain or cord of cells in the testes as in the ovaries, I cannot tell, owing to the many convolutions, but think there are at least several such chains or cords. The regular growth of these causes the testis to be divided into zones. During the growth period the cells grow to the size of the spermatogonia and cannot be distinguished from them save by their position in the testis (growth zone). When preparing for division the cells lose their linear arrangement; the chromatin forms eight double rods which lie close together (synapsis stage). Each double rod becomes ring shaped and the rings contract until only a small lumen is left. An additional pair of constrictions converts the ring into a tetrad. The division forms two secondary spermatocytes, each with eight diads, and another division immediately following forms four spermatids, each with eight chromosomes. The spermatids are grouped in fours. In some of these groups, each of the cells becomes filled with an achromatic substance which presses the chromatin and protoplasm against the cell wall. This substance increases greatly in quantity and becomes more and more like yolk (called Austreibestoff by C. Heider, '79). In very rare cases in some very young spermatids I have seen a small achromatic sphere in the cytoplasm and have found a still smaller sphere in the cytoplasm of some primary spermatocytes. On the other hand the substance in question at first appears to lie within the nucleus and is closely surrounded by chromatin. A plausible explanation might be that the small globule seen in some very young spermatids moved up to and indented the nucleus, there growing until it became larger than the original nucleus yet I have been unable to find steps in such a process, in fact I believe the substance arises in the nucleus.* The cells containing these spheres nourish the spermatozoa, and may be called nutritive cells.

Going back to the spermatids, many groups degenerate, becoming much shrunken, while those that will form spermatozoa collect into larger groups, the cells of which begin to elongate radially. As the spermatids become longer they come to lie nearly parallel. The nucleus elongates into a spindle shaped deeply staining fiber, covered by a thin layer of achromatic substance. The remaining protoplasm fuses with that of adjacent spermatozoa and forms a mass in the center of the group. These groups sometimes lie with one end against a nutritive cell, and when the groups break up the spermatozoa collect around nutritive cells until the cytoplasm of the latter disintegrates and the chromatin collects into rounded masses that float about, leaving only a sphere of a yolk-like substance, the achromatic substance, which has now developed an affinity for plasma stains. As the elements pass down the vas deferens the nutritive spheres lie near its walls and on reaching the spermatophore the nutritive spheres (Austreibekörperchen) form a layer several spheres deep, and become pressed together into polyhedrons. Most of the chromatic spherules form a layer within the nutritive layer but some chromatic spherules caught between the nutritive spheres, become elongate curved bodies that remain for a long time. The spermatozoa fill the center of the vas deferens and lie parallel to one another. In the spermatophores they extend radially from the nutritive walls. In spermatophores that have been attached to the female a long time, the nutritive material has disappeared, leaving a mass resembling evacuated cell walls.

---

*Compare formation of "Glanzkörper" from the plasmosomes of Pelomyxa: Goldschmidt, Arch. f. Protistenkunde 5, p. 130.

The same description of the spermatogenesis holds in general for *Pandarus sinuatus*, though there are many minor differences. As regards the behavior of these nutritive bodies I have observed one similar instance, in *Peripatus*, but in *Peripatus* the nutritive bodies are nuclei of degenerating cells. In these copepods the nutritive body appears to form in or in close relation to the nucleus, but too little is known both of the nutritive bodies in *Peripatus* and copepods to suppose them genetically related.

VI. Polarity of the Egg.

The fertilized egg and embryo in all cases in which the egg string extends straight behind the animal and contains a single linear series of eggs, is definitely oriented in relation to the mother. The animal pole of the egg is posterior and the first protoplasmic cell and resulting head end of the embryo is latero-ventral. The chief axis of the egg is manifested in the ovary in the flattening of the egg (being the shortest axis). In any stage in which the primary oöcyte is considerably thicker than the diameter of the nucleus, the latter lies nearer the animal pole. The head end of the embryo coincides with the region in which the first protoplasmic cell is formed, and this is probably determined by the point of entrance of the sperm. The seminal receptical opens into the oviduct by a small orifice which would lead sperm to the egg at or near the position of the future head of the embryo. Cases of rotation of the long axis of the embryo, which sometimes occur, might be due to the spermatozoön getting around the egg before entering.

The first polar body is extruded in a slightly eccentric position on that flat side of the egg directed toward the free end of the egg string. Thus the chief axis of the egg does not exactly coincide with the shortest axis, but is a little inclined toward the anterior end, yet not enough to cause the first polar body to lie in the first cleavage furrow. This is probably due to the great inequality in the first cleavage. Furthermore, the blastopore does not close at a point diametrically opposite to the first polar body, but considerably posterior to such a point. From a study of the literature on gastrulation in crustacea, I am led to believe that the blastopore in the majority of cases in this group closes posterior to the vegetal pole and such a character would be accentuated by flattening of the egg. As the eggs occur in single linear series in an egg string surrounded on all sides by sea water, there is probably nothing in the surroundings that could determine the axes of the egg, and we should regard them as probably determined by the structure of the protoplasm.

VII. Summary.

1. In the maturation of the eggs of parasitic copepods the behavior of the chromosomes in regard to the question of *reduction* is very similar to the same process in the free living copepods, yet I differ from Haecker as to the reducing division, considering the first maturation division most probably to be the reducing division.

2. In the spermatogenesis only a small proportion of the spermatids become spermatozoa. Many spermatids degenerate, others become metamorphosed into peculiar nutritive cells. The protoplasm of the nutritive cells degenerates leaving only a sphere of deutoplasm "Austreibekörperchen," which C. Heider ('79) thought was a glandular secretion.

IX. Abbreviations.

n = Nucleolus.
p = First polar body.
s = Sphere of protoplasm, probably an abnormal structure
x = Darkly staining cell in yolk.
y = Yolk spherule.

EXPLANATION OF PLATE I.

(*Figs. 1–14, The Dichelestid.*)

FIG. 1. An Oögonium.

FIG. 2. Three consecutive primary oöcytes in the synapsis stage. Optical section of the cord of ovarian cells.

FIG. 3. Early growth stage of same, partial disappearance of cell walls.

FIG. 4. Middle portion of oöcyte containing the nucleus, from longitudinal section of oviduct. Commencement of formation of yolk spherules (*y*). Two nucleoli are seen (*n*).

(*Figs. 5–10, Pandarus sinuatus Verrill.*)

FIG. 5. A little later stage. Nucleus viewed from the animal pole. Two of the eight double rods, or diads, are joined together end to end (to the right), so that it is difficult to distinguish them separately.

FIG. 6. The same in a section through the short axis containing six of the diads. Above at *n*, the nucleolus of another egg is represented to show a later stage in the disintegration of the nucleolus.

FIG. 7. The first maturation spindle.

FIG. 8. Later stage of same, in which it has rotated through almost a right angle and is pushing through the egg membrane to extrude the first polar body.

FIG. 9. Late metaphase of the second polar spindle. The first polar body is represented at *p*. (Constructed from two sections of the series.)

FIG. 10. The same stage from a surface preparation of the egg. The wall of the oviduct was torn off and the eggs allowed to separate in sea water (two hours). In separation the first polar body is pulled out into a stalked structure.

(*Figs. 11–17, The Dichelestid.*)

FIG. 11. Less magnified figure of prophase showing sphere of cytoplasm (*s*) near periphery of egg.

FIG. 12. A later stage. The nuclear sap has faded, it is vacuolated, and around each chromosome stains darker (*f*) than elsewhere. The two chromosomes that are stippled are out of focus. The chromosomes are ring-shaped tetrads.

FIG. 13. The chromosomes are each enclosed in a linin sac, and these sacs have begun to elongate.

FIG. 14. The same or a little later stage. The elongated linin sacs lie parallel, with spindle fibers developed between them. The incipient spindle is formed in dense (dark) protoplasm while this latter is surrounded by looser (paler) protoplasm.

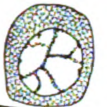
FIG. 15. Equatorial plate, same stage as 14.

FIG. 16. The spindle has elongated perpendicular to the short axis of the egg. The dense protoplasm forms the two poles of the spindle. While the loose protoplasm forms astrosphere-like structures enclosing the poles of the spindle.

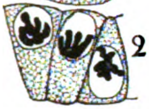
FIG. 17. The spindle is half rotated toward the short axis of the egg and has considerably shortened in doing so.

FIG. 18. *Læmargus muricatus* Kröyer. Central portion of the egg containing the first maturation spindle. Metaphase. The spindle fibers do not show in this preparation. Three of the eight chromosomes are in focus, one being seen from the end and appearing smaller than the others.

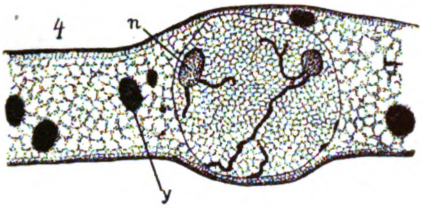
1

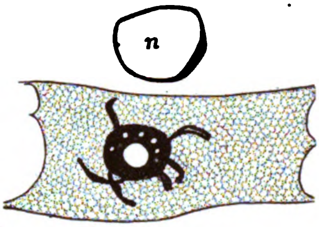


2

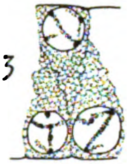


4

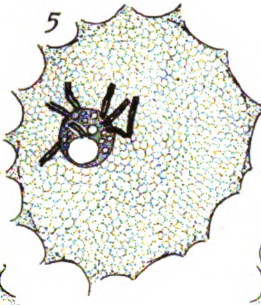




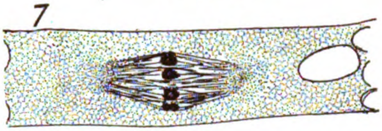
3



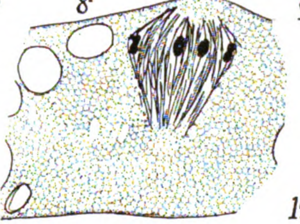
5



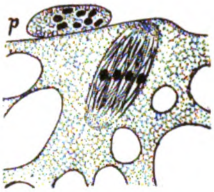
7



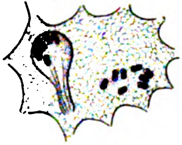
8



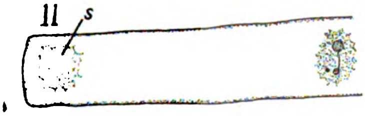
9



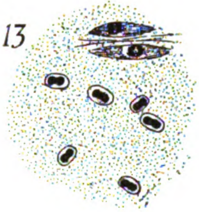
10



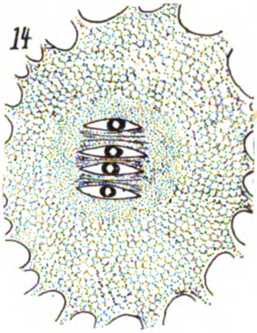
11



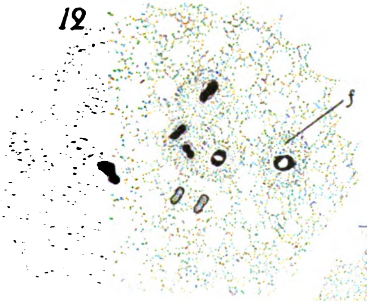
13



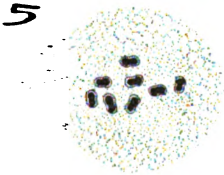
14



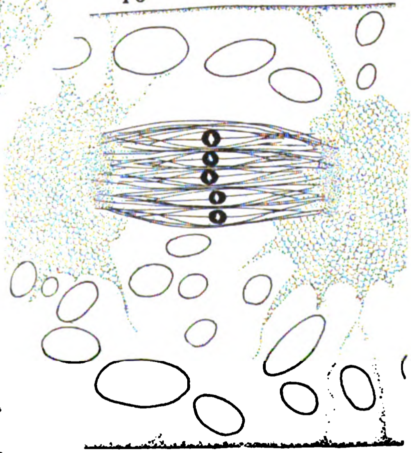
12



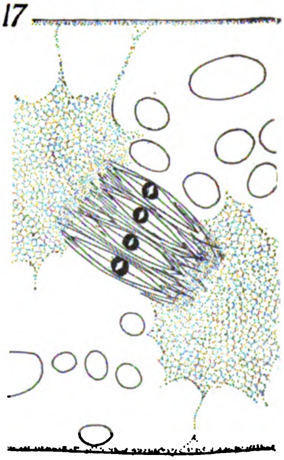
15



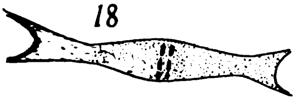
16



17



18



[Reprinted from BIOLOGICAL BULLETIN, Vol. XII., No. 2, January, 1907.]

ON THE DEVELOPMENT OF PARASITIC COPEPODS.[1]

PART II.

J. F. McCLENDON.

CONTENTS.

I. Cleavage of the egg 57
 1. *Læmargus muricatus* 57
  A. First cleavage 57
  B. Second cleavage 58
 2. The Dichelestid 60
  A. First cleavage 60
  B. Second cleavage 60
  C. Third cleavage 60
  D. Fourth cleavage 60
  E. Fifth cleavage 61
  F. Sixth cleavage 61
II. On the nature of the cleavage process 62
III. The mesoblast 63
 1. The nauplius mesoblast 63
  A. The germ cells 63
   a. *Pandarus sinuatus* 63
   b. The dichelestid 65
  B. Mesoblastic rudiments of the nauplius appendages 65
 2. Post-nauplius mesoblast 67
IV. The entoblast 68
V. Polyspermy 69
VI. Relation of pressure, polyspermy, etc., to the type of cleavage 70
VII. Summary 74
VIII. Explanation of plates 75
IX. Bibliography 76

The egg is covered by a chitinous chorion and the eggs are packed (and much compressed) in a tough chitinous tube (*e. c.*, Text Fig. 1), which is attached to the mother. In order to allow removal from the tube, the eggs must be fixed in fluids which cause them to draw away from the chorion (partly by increasing the osmotic pressure inside the chorion) and which do not make them friable. The only fluids which served contained alcohol and nitric acid. Nitric acid of 5 per cent.–10 per cent. in alcohol of about 30 per cent. served well enough for cell lineage but allowed the chromosomes to swell (partly re-dissolve in water?). The addition of chromic acid (Perenyi's formula) prevented the swelling of the chromosomes and made a good fixitive if manipulated properly. The chromic acid in this mixture is changed to blue chromic oxide, and P. Mayer in the first German Edition of Lee's "Vade Mecum,"[1] says it contains 30 per cent. alcohol, 5 per cent. nitric acid and a little nitric ether and chromic oxide, the last two having no effect in fixation. However, it has been my experience that the chromic oxide was necessary to prevent swelling of the chromosomes. Fischer ('99) says that nucleinic acid is not precipitated by dilute nitric acid, and that its precipitate formed by alcohol is soluble in water. It is possible that the swelling of the chromosomes is due to the solution of nucleinic acid or its compounds in the aqueous staining bath. In addition to its other qualities, nitric acid bleaches the eggs of *Pandarus*, and others that contain pigment, and although it is difficult to wash out (in 70 per cent. alcohol) it was found to be an indispensable ingredient.

---

[1] Thesis accepted by the Faculty of the Department of Philosophy of the University of Pennsylvania toward the degree of doctor of philosophy.

I tried various standard fixatives for eggs to be sectioned, and found them little better than Perenyi's fluid; but for whole adults to be sectioned for the ovaries, etc., the latter fluid seemed to be much inferior to some others. It was probably too much diluted by the body fluids.

For staining whole eggs Delifield's hæmatoxylin diluted and acidulated (Conklin's formula) was the most convenient, but for sections various stains were used. In Hermann's safranin-gentian violet, besides the usual differentiations of chromatin, the centrosomes (centrioles) stained red and the archoplasm blue (unless the sequence of stains was reversed). Iron hæmatoxylin gave the sharpest stains for chromosomes.

It is probable that there can be no fixation without some artefacts, and that the differentiation shown by many stains is due to differences in the size and density of particles of protoplasm coagulated by fixation as claimed by some writers. I have paid special attention to the structure of coagulated proteids, and repeated experiments of Fischer and others by producing granular and "curdled" precipitates, and aster-like formations in albumin, and by staining the same. These led me to believe that probably none of the finest structures seen in the fixed protoplasm could be relied upon as representing structures in the living cell, but that such large bodies as chromosomes, spheres, etc., could not be considered artefacts, though their finer structure may be changed.

---

[1] "Grundzüge der Mikroskopischer Tecknik," by Lee and Mayer, Berlin, 1898.

In the cell lineage I have used the quartet system of nomenclature (of Kofoid, '94) as applied by Bigelow to *Lepas* (the only crustacean whose cell lineage has been described beyond the 16 cell stage) to facilitate comparison among crustacea, but do not think this type of cleavage closely related to that of annelids and molluscs, in fact the cleavage of the parasitic copepods does *not* follow a quartet system and I hope no one will be misled by the inappropriate nomenclature.

The cells of the 4 cell stage are designated *a*, *b*, *c*, *d* in a dextral order, *a* being the left anterior cell. An exponent denotes the order of the generation starting with the ovum as the first. A second exponent is used to distinguish a cell from other cells of the same generation and derivation. The odd numbers refer in cases of equatorial division to cells nearer the vegetal pole; of transverse, to cells nearer the anterior end; of longitudinal, to cells nearer the sagittal plane, or in case the cleavage coincides with that plane, the right side. Thus *equatorial* refers to the equator of the chief axis of the egg while *sagittal* and *transverse* to the axes of the embryo that will develop therefrom, but which may be distinguished in the egg as early as the 2 cell stage.

To determine the second exponent of the two daughter cells of any cell division, multiply the second exponent of the mother cell by two and the product is the second exponent of that daughter cell which has an even number for this exponent, and is one greater than the second exponent of the daughter cell which has an odd number for a second exponent.

For cell lineage, whole eggs had to be used, and it was exceedingly difficult to get them out of the egg tube. The best way is to separate the eggs by cutting the tube between them with a sharp "spear head" dissecting needle under the microscope, which increases in difficulty in proportion to the flattening of the eggs. When the eggs are separated the polar bodies are lost and other means of orientation are necessary. In stages before the origin of the primary germ cell it was necessary to lay the eggs on the slide with a determined pole uppermost. The eggs, except when abnormally placed, have the vegetal pole turned toward the mother, and by placing the mother and attached egg strings in cedar oil on a slide under a Zeiss binocular dissecting microscope, it was possible to lay the eggs with vegetal pole up as they were separated, place a cover glass over them to prevent turning, and run balsam under from one side.

Schimkewitz ('96, '99) concluded that pressure was an important factor in determining the form of the cleavage of parasitic copepods.

Pedaschenko ('93, '97, '98) worked for a number of years on the embryology of *Lernæa branchialis* and traced the cell lineage to the 16 cell stage, but was mistaken in the orientation, thinking the first protoplasmic cell to be formed at the animal pole and not distinguishing between the two flat surfaces (dorsal and ventral) of the egg in early stages. He found the germ cells to arise from four cells at the edge of the blastopore and considered two of these to be male and two female. The identity of two of these cells was lost (incorporated in the other two) and the remaining two gave rise to the sex glands. If such were the case, it seems to me that we should expect frequent occurrence of bilateral androgyny (hermaphroditism). The close relation of Lernæa to the forms I studied has made Pedaschenko's work of great service as a hand-book.

Grobben ('79) had long before found the germ cells to arise from four cells of the anterior lip of blastopore of the phyllopod, *Moina*.

C. B. Wilson ('05) includes in his excellent monograph of the Caligidæ, a description of the general embryology of these parasitic copepods.

The only crustacean whose cell lineage has hitherto been carried beyond the 16 cell stage is *Lepas*, as described by Biglow ('02). Pedaschenko pointed out the resemblance between the segmentations of *Lepas* and parasitic copepods and I believe this resemblance is fundamental. The endoblast arises one generation earlier in *Lepas* than in parasitic copepods but this may be due to larger amount of yolk in the latter, which causes a retardation in the segregation of organ-forming substances, and of gastrulation.

Canu ('92) published a paper which I have not seen, which included embryology of copepods. Further consideration of the literature may be found in the text.

I. Cleavage of the Egg.

1. *Læmargus muricatus* Kröyer.

A. First Cleavage. — At the earliest stage I have (Fig. 19) the male and female pronuclei lie side by side at the center of the egg and at the equator of the spindle. At this stage the egg throws out a number of yolk spherules into the space between the egg and the chorion, but the exact nature of this process seems obscure. Each pronucleus contains a nucleolus, and the chromatin is being aggregated into chromosomes. The pronuclei are of the same size and apparently similar in every respect. There is a deeply staining centriole at each pole of the spindle, surrounded by a layer of hyaloplasm that is drawn out into astral rays connected with the surface of the egg, and mantle fibers connected with the pronuclei. The astral rays of one pole are thicker (stronger) than those of the other. Where the mantle fibers come in contact with the nuclear membrane, the latter is pushed in (and partially dissolved?) and finally becomes dissolved, and the mantle fibers become attached to the chromosomes. The spindle thus formed is elongated. The astral rays of one pole shorten more rapidly than those of the other, drawing this pole nearer one edge of the egg than the other. The hyaloplasm is drawn from between the yolk globules and the astral rays increase in thickness. Not only is the hyaloplasm drawn into the astral rays, but small lumps of hyaloplasm adhere to their surfaces and move toward the centrosomes. Thus the spheres increase in size by thickening of the hyaloplasm layer around the centrosome and become surrounded by protoplasm drawn in along the rays. One sphere grows larger than the other and moves to the surface of the egg. Some yolk granules are caught between the astral rays and form a clear space between the sphere and the egg membrane and push the astral rays outward. We thus have a central space almost free from rays, surrounded by an annular area in which the rays are especially aggregated. The center is bulged out and the annular area sunken in by the stress (Fig. 20). Some astral rays connect with those of the other pole, forming "spindle fibers" outside the mantle fibers. The sphere at the surface of the egg soon pulls all the hyaloplasm from between the yolk granules in that half of the egg and the astral rays that pass through the yolk break and are drawn into the sphere. The mantle fibers connected with the outer pole shorten more than those of the opposite pole, and the equatorial plate moves to the plane of the ensuing cell division. The cell division is very unequal, separating a cell containing very little yolk (*ab*^{1}) from one containing practically all the yolk (*cd*^{1}, Fig. 21).

Going back a little, by the dissolution of the nuclear membranes a good deal of nuclear sap is liberated. This fluid is hard to follow, but I have some slides that seem to show that most of it goes toward the sphere that reaches the surface, and is therefore included in the cell *ab*^{1}. The first cleavage plane is parallel to the chief axis, but is very eccentric because of the great inequality of the division. (See the section on orientation of the egg.)

B. Second Cleavage. — After fusion of the chromosomal vesicles in the two cell stages (Fig. 21), the nuclei thus formed remain connected for a short time by interzonal fibers. I have no stages in the division of the centrosome, but soon after this division the two centrosomes are at the ends of a spindle shaped sac or centrodesmus (Fig. 21, small figure to right below). Mantle fibers become attached to the nuclear membrane and the chromosomes gather in that side of the nucleus nearest these points of attachment. From this stage on, the histories of the protoplasmic cell and the yolk cell are different and will be treated separately.

The nuclear membrane of the protoplasmic cell dissolves in the region of attachment of the mantle fibers, which then become attached to the chromosomes. The remainder of the nuclear wall, and the nucleolus dissolve and the chromosomes are arranged in the equatorial plate (Fig. 23), which is at first far removed from the central spindle, but later the central spindle assumes its normal position in the center of the peripheral spindle (Fig. 24). The division divides the cell by a meridional (sagittal) cleavage into equal daughter cells (a^3 and b^3).

In the yolk cell, as the attraction spheres separate they grow in size (Figs. 21–24). The central spindle presses against the nucleus, forming a groove (Fig. 22) which makes it appear as though the nuclues was divided (maternal and paternal elements distinct), but sections show that this division does not pass completely through the nuclues. The nucleus is drawn out to about four times its original length (Fig. 23), one sphere moving faster than the other and reaching the surface of the egg, on the right side of the protoplasmic cell.

This elongated nucleus is bent considerably and suggests that it is being elongated by a force applied internally, and is bent by external resistance, but I think the bending may be due to the unequal pressure of the yolk, and the elongation of the nucleus may be due wholly or in part, to the contraction and separation * of the mantle fibers. The nuclear membrane dissolves, and the equatorial plate is formed (Fig. 24). It is to be noted that whereas the elongated nucleus is bent the fully formed spindle is straight. In Fig. 23 it is seen that the end of the nucleus attached to the peripheral sphere is enlarged and nearer to its sphere than the other end is, probably due to increased tension of the mantle fibers at this end, accompanied by pressure of the yolk on the sides of the nucleus.

On dissolution of the membrane all the nuclear sap goes into the peripheral sphere. A yolk spherule is often caught between the astral rays and the cell wall (Fig. 24, c^3). Protoplasm migrates along the astral rays to the spheres. The division cuts off a small protoplasmic cell from a large cell containing practically all the yolk (d^3). I have not worked out the cell lineage any further in this species, though it appears to be essentially the same as the dichelestid.

---

* By elongation of the central spindle?

2. *The Dichelestid.*

A. First Cleavage.—The earliest stage I have of this is an anaphase of the first cleavage (Fig. 25). It is similar to the same stage in the preceding species save that the centrosomes it they exist at all are larger and less dense, and the sphere reaching the surface collects a considerable mass of cytoplasm around it. The cleavage plane is "meridional" or more correctly, it is perpendicular to the equator of the egg, but owing to the great difference in size of the protoplasmic and yolk cells thus formed, it does not pass through the animal or vegetal pole (Fig. 26).

B. Second Cleavage.—The yolk cell (cd^2), is sometimes retarded in division—in Fig. 26 its nucleus is yet a mass of chromosomal vesicles while that of the protoplasmic cell (cd^2) has reached a late prophase. Already a thickened layer of protoplasm marks the place where c^3 will be cut off.

The protoplasmic cell (ab^2) divides by a meridional (sagittal) furrow into two cells, a^3 and b^3, almost equal in size (Fig. 27). The yolk cell produces an elongated spindle similar to that in the preceding species, one pole of which reaches the surface of the egg to the right (left, when viewed from the vegetal pole) of b^3 (Fig. 27). The protoplasmic cell that is cut off (C^3) often contains a considerable quantity of yolk (Fig. 28). Already a thickened layer of protoplasm (Fig. 33) marks the place where $d^{4.2}$ will be cut off.

In this and the two succeeding cleavages, the poles of the yolk cell spindle are differentiated by the appearance of larger granules on the astral rays of the posterior side of the sphere that is to remain in the yolk (Fig. 27). This probably occurs also in the first cleavage but I have not the right stage to show it. These granules are probably homologous to lumps of cytoplasm on the astral rays of *Læmargus muricatus.*

C. Third Cleavage.—The division of the protoplasmic cells a^3, b^3, and c^3 is equatorial (parallel with the face of the disc) (Fig. 28). The yolk cell gives off a protoplasmic cell, $d^{4.2}$ to the left of a^3. Large granules appear on the astral rays of the posterior side of the sphere left in the yolk. A thickened layer of protoplasm marks the place where $d^{5.2}$ will be cut off.

D. Fourth Cleavage (Figs. 29–30).—In this cleavage the division of the yolk cell ($d^{4.1}$) cuts off a protoplasmic cell $d^{5.2}$ (Fig. 31) to the right of the cap of protoplasmic cells; $d^{4.2}$ divides equatorially, $C^{4.1}$ and $b^{4.1}$ transverselsy; $a^{4.1}$ divides obliquely but the daughter cells ($a^{5.1}$ and $a^{5.2}$, Fig. 31) come to lie one behind the other.

On the dorsal side $a^{4.2}$, $b^{4.2}$, and $C^{4.2}$ divide transversely.

Fig. 30 is an enlarged view of the spindle in the yolk cell, constructed from the two consecutive sections. The astral rays are only partially shown, and, connecting them, the alveolar (or reticular ?) hyaloplasm between the yolk spheres is represented by dotted lines. This is a little later stage than Fig. 29 and the spindle has shortened more. The distinctness of the centrosomes is exaggerated in the figure, in fact it is doubtful whether we deal here with centrosomes, but that the sphere is denser in the center can be shown in some cases with Hermann's safranin-gentian violet stain.

E. Fifth Cleavage (Figs. 31–35). — Of this cleavage I have not enough stages to be sure of the lineage of every cell. There are many disarrangements due to the cells extending over the yolk and slipping on one another, which makes their lineage extremely difficult to follow. In the figures I have divided the derivatives of *a*, *b*, *c* and *d* by heavy lines, and by comparing Figs. 31 and 32, one can see the great change that has come about.

In this cleavage the yolk cell divides, giving off (near the center of the ventral side) the last protoplasmic cell $d^{6.2}$ (Fig. 32), which is the primary germ cell.

F. Sixth Cleavage. — In the fifth cleavage the divisions were not synchronous, in the sixth cleavage the division of two cells, $d^{6.2}$ (the primary germ cell) and $d^{6.1}$ (the primary entoderm cell) is delayed until some of the other cells are dividing for the eighth time. After cleavage of the majority of the cells (Fig. 33) the blastoderm stretches over the yolk until it has half covered the latter (Fig. 34). During this process some derivatives of *d* at each side of the egg and at the end of the blastoderm, come to lie under the others and give rise to mesoderm. The yolk cell (entoderm) divides totally by a sagittal furrow (Figs. 34, 35) and the primary germ cell sinks beneath the blastoderm and divides by a sagittal furrow into two cells of unequal size.

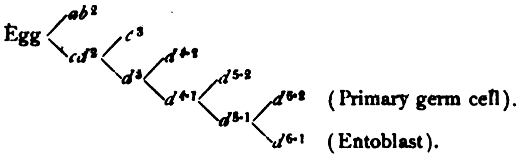


TABLE OF CELL LINEAGE TO 32 CELL STAGE.

II. ON THE NATURE OF THE CLEAVAGE PROCESS.

Since Van Beneden in 1883 put forward the hypothesis that separation of the chromosomes and division of the cell was caused by contraction of the fibers of the karyokinetic figure, the question of the mechanics of mitosis has aroused a great deal of interest. The large size and peculiar form of the spindles in the early cleavage of this egg, make them favorable objects for observations on this point. I have attempted to harmonize observations on the structure of coagulated colloids, and the modern theory of the ultramicroscopic structure of colloids, with the observations on artificially produced asters in colloids, and asters and spindles appearing during mitosis in living cells. A theory of Rhumbler and others as to the mechanics of the formation of asters seems in general to be the only one applicable to the observations I have made, yet I do not believe that this theory is inseparable from the alveolar theory of the structure of protoplasm. Asters can be produced in colloids which we have no reason to believe have the alveolar structure in the strict sense. According to Mann ('06), colloids consist of minute or ultramicroscopic particles suspended in a thin fluid. On congealing (Hardy, Jour. of Physiol., V., 24), these particles by mutual attraction form rows which make up a meshwork (or interalveolar structure?) giving consistency to the mass. When colloids are coagulated with substances (electrolytes) that act strongly and quickly, the particles are large enough to be seen with the microscope and are at first distributed homogeneously through the fluid, but soon arrange themselves in rows which make up a meshwork ("gerinnselbilder" of Fischer). This passing of a colloid from the "sol" to the "gel" or congealed state may be hastened by addition of a fragment of coagulated colloid to the former, in which case the rows of drops or particles arrange themselves radially around the fragment, and an aster is formed. Fischer ('99) varied this experiment in a number of ways, and one of his experiments modified slightly, might be tried by every one interested in the subject without much trouble: Spread a layer of egg albumin (which consists chiefly of albumin and a little globulin) on a slide and through a capillary tube introduce a small drop of a fixing solution into the albumin, observing the changes that take place under the microscope. The albumin immediately around the drop is coagulated into a membrane through which the fixing solution diffuses and from which radiations begin to form, giving the whole structure the appearance of an aster with the drop of fixing solution and the membrane around it as the centrosome. If the rays form, as they seem to, by mutual attraction of the drops or particles in the fluid, such rays or rows of drops would exert a pulling force, and if their ends were released should shorten by synæresis into a spherical mass. This may be the nature of the fibers of the karyokinetic figures in the cleavage of these copepods but does not explain the direction of movement of the asters.

III. Mesoblast.

1. *Nauplius Mesoblast* (*Pandarus sinuatus*, Pl. IV. and V.).

When the cap of the protoplasmic cells has covered about one third of the yolk some of the marginal cells (lip of the blastopore) become differentiated as mesoblast. Of these one or more on the right and left edge will give rise to mesoderm of the first and second antennæ, and one near the middle of the ventral side and distinguished by its large nucleus (Fig. 37) will give rise to the sex or germ cells.

A. The Germ Cells. — This primary germ cell is turned under the rim of the blastopore (Fig. 38) and divides by a sagittal furrow into two (Figs. 39–40), which lie about the center of the ventral side just under the ectoderm. About the time of the closure of the blastopore these two divide by transverse furrows into four (Fig. 41). This group of four cells rotates until one cell is anterior, two lateral and one posterior. (In Fig. 42 the rotation is not quite completed.) But there is considerable variation in the amount of rotation (Figs. 41–48). The four germ cells lie just beneath the ectoderm until the *Metanauplius* stage, when by concentration of the ventral nerve chain, the latter is pushed under them and they rest on top of it (Fig. 56). I have said the germ cells have large nuclei — the nucleus is further characterized by the fact that the chromosomes remain distinct as oval masses just inside the nuclear membrane (Fig. 52). The chromosomes can be counted and are sixteen, just twice the number in the female pronucleus. The cytoplasm is much vacuolated. The germ cells are flattened against each other, and are flattened against the ectoderm in the early stages (Figs. 49, 50). In later stages they detach from the ectoderm, and round up (Figs. 52, 56). And still later (during the *Metanauplius* stage from 24 to 72 hours after hatching of the larva) they separate and pass laterally and upwards into the yolk and two of them come together dorsal to the intestine, and I have not traced them further than the fourth day after the larva hatched, when they were still two in number. Pedaschenko says that two of the four genital cells pass to the right and two (one lateral and one median) to the left. Each pair fuse and, probably by degeneration of one nucleus, becomes a single cell, which finds its way upwards and posteriorly and by division forms the ovary of that side. The fusion of each pair he considers of great significance and the basis of a theory on the origin of the sex of the adult. He believes that one cell of each pair is male and one female and the one whose nucleus persists after fusion of the cytoplasm determines the sex of the animal. His belief that two cells of the four are male and two female is based on comparison with O. Hertwig's account of *Sagitta* in which this condition exists, with difference however in the later history. In *Sagitta* the two female cells give rise to the ovaries (in the anterior part of the animal) and the two male cells give rise to the testes (in the posterior part of the animal).

Sex is said to be determined in some animals by amount of food (of the individual, the parents, or the grandparents) in others by fertilization *vs.* parthenogenesis, in others by dimorphism of egg or spermatozoön, in others by temperature, etc. Pedaschenko proposes an additional factor.

Haecker ('97) found in *Cyclops* the primary germ cell differentiated from the somatic cells at the close of the fourth cleavage or one generation earlier than in the parasitic copepods.

Boveri ('92) in *Ascaris*, and Haecker ('97) in *Cyclops* traced the "Keimbahn" from the first cleavage. In *Ascaris* the visible difference between germ cell and somatic cell was in the chromosomes, in *Cyclops* in the cytoplasm. Early differentiation of the germ cells has been noticed in a large number of animals, but the causal factors in their differentiation are yet unknown. From Boveri's account of *Ascaris*, it seems that the cells of the "Keimbahn" preserve all the characters of the fertilized egg, while the somatic cells lose some characters. Yet the mature ova and spermatozoa of most animals are possibly as highly differentiated as any somatic cell.

In the dichelestid the germ cells have the same origin as in *Pandarus sinuatus* but they differ in appearance. Fig. 57 shows the primary germ cell beginning to be turned under the blastoporal rim. Fig. 58 shows a stage after the division of the germ cell into two cells (of unequal size). If we followed Pedaschenko's theory we might consider the large cell as female and the small cell as male as it is always true that one is larger than the other. The nuclei of the two germ cells lie in their ends that are nearest the free border of the blastoderm (blastopore). These two cells divide into four and the nuclei of two are larger than of the other two, but the cell boundaries between them are extremely difficult to make out.

B. The Mesoblastic Rudiments of the Nauplius Appendages (*Pandarus sinuatus*) arise from cells turned under the rim of the blastopore during epibole. When the cap of protoplasmic cells has covered about one third the yolk (Fig. 38) a few cells are turned under the rim at the extreme right and left, that is to say at the edge of the disc shaped egg. These cells are the mesoblastic elements of the first and second antennæ and divide on each side into two masses (Fig. 40, an^1, an^2). The time of this division varies slightly, the elements being sometimes widely separated before closure of the blastopore (Fig. 40) and sometimes close together just after the closure of the blastopore (Fig. 41, an^1, an^2). Just before closure of the blastopore, a few cells are turned under its lip on each side (Fig. 40, *md*) and are the mesoblastic rudiments of the mandibles. This completes the rudiments of the nauplius appendages. After the close of the blastopore the post nauplius segments are laid down by teloblastic growth at the posterior end, and the nauplius is pushed (compressed) forward, carrying the rudiments of the second antennæ and mandibles forward (Figs. 47–48), and causing the three pairs of appendages to lie closer together. In stage *D* (Figs. 45–6) the appendages begin to grow out and at the same time the muscle cells elongate into fibers. I think it more profitable to follow these latter backward in development, as it seems doubtful whether they have a single or a double origin. Observe the muscle cells in Fig. 45 elongating radially and attached peripherally to the rudiments of the appendages. In Fig. 44 (Stage *C*) the muscle cells (one shown at *m*) are just beginning to differentiate from the mesoblastic rudiments of the appendages, and two of them have begun to elongate (compare Fig. 50, *m*). The question arises whether all or only some of these muscle cells arose from the mesoblastic rudiments of the appendages.

Just after the closure of the blastopore a few cells similar to these muscle cells are seen considerably removed from the mesoblastic rudiments of the appendages (Fig. 41, *m*). And just before closure of the blastopore minute cells with scarcely any cytoplasm are seen budding off from the ectoderm in this region, (Figs. 40, 49, *x*). There is a slight probability that some of the cells *m* arise by growth of the cells *x* which would be a case of muscle cells arising from ectoderm as in cœlenterata, etc. But small cells with hardly any cytoplasm are found in the yolk at many stages of the embryo (Figs. 44 and 47, *x*) and although I have not closely traced them from cells like *x* in Fig. 40, I think their resemblance in structure indicates a likeness in origin. I think the evidence indicates that all the mesoblast arises from cells turned in from the lip of the blastopore, as is the case in other copepoda, phyllopoda, decapoda and cirripedia.

The muscle cells when first elongated push the ectoderm toward the center and mass it in a sort of structure which somewhat resembles the "dorsal organ" which disintegrates, and the elements of which wander into the yolk. The muscle cells are thus arranged radially just beneath the extremely thin dorsal ectoderm (Fig. 45) but the forward movement of the appendages carries their peripheral ends forward (Figs. 45–48) until they assume a longitudinal direction. Contractile fibrillæ begin to form in the muscle cells in stage *E* (Fig. 47) and the nucleus and undifferentiated cytoplasm is pushed to one side. In the liberated nauplius the muscle fibers run almost the whole length of the animal and show cross striations (Fig. 51). Each appendage then has at least one muscle fiber attached to the anterior and one to the posterior border of its base. Muscle cells that go into the hollow appendages as they grow out, form muscles attached to the bifurcated ends of the appendages (Fig. 51, left side).

The same description in general holds good for the dichelestid and *Læmargus.* In these the mesoderm of the appendages is clearly derived only from marginal cells. In *Læmargus* the ectoderm massed in the middle of the dorsal side by growth of the dorsal muscle fibers forms a more conspicuous "dorsal organ" than in the other species and the elements arising from its disintegration are more numerous.

In relation to the formation of the appendages might be mentioned the segmentation of the nauplius of *Læmargus.* Soon after the closure of the blastopore the embryo is divided by bands of thinner ectoderm into three segments corresponding to the three pairs of nauplius appendages. This segmentation slowly disappears with the development of the nauplius. Other species show it but to a less degree than *Læmargus.* This segmentation might be used as evidence that the nauplius of ancestors of crustacea was segmented or it might be considered as cœnogenetic and associated with the development of the appendages and neuromeres of the nauplius.

2. *Post nauplius mesoblast* (*Pandarus sinuatus*).

At the closure of the blastopore some of the marginal cells are turned in (Fig. 49, *Mp*) and become the mesoblast of the post naupliar segments. These cells are much larger than the surrounding cells (Fig. 41) and form a mass at the posterior end of the animal that is destined to develop mesoblastic somites by teloblastic growth. By rapid division the cells become small and by this time the ectoderm has completely closed over them (Fig. 42). This mass of cells divides in the sagittal plane into two masses (Fig. 44), which begin to grow forward as a pair of broad bands under the neural thickenings of the ectoderm (Figs. 46, 47, *Mp*). From the anterior ends of these bands oval masses are cut off that are the mesoblastic somites (Fig. 48).

IV. Entoblast.

The entoblast is segregated one generation later than in Lepas. In the 32 cell stage the entoblast consists of one cell that contains practically all the yolk and which does not divide until the majority of the cells have completed the seventh cleavage and some are in the eighth. It then forms a very long transverse spindle with the poles inclined anteriorly. The daughter nuclei are widely separated, but in *Pandarus*, *Læmargus* and other Caligidæ the yolk does not segment. The next (second) division occurs about the time the "blastoderm" has covered half the yolk. The spindles extend longitudinally and the poles are inclined outward. Each of the two spindles is shorter than that of the previous division (Figs. 35, 38). The next (third) division occurs about the time of the closure of the blastopore (Fig. 40). There is much variation in the direction and curvature of each of the four spindles, but the daughter nuclei are about equally distributed through the yolk as they are after each division. The fourth division occurs in stage *B* (Fig. 42) and the fifth in stage *C* (Fig. 43).

In *Eudactylina* the yolk segments in the first three cleavages of the entoblast, (forming eight cells) after which the entoblast forms a syncytium. In the dichelestid the yolk divides into four cells and is then transformed into a syncytium. In the remaining species studied a syncytium is formed from the first. This omitting of the cleavage of the yolk is probably not entirely due to the amount of yolk present, which is as great in the dichelestid as in *Læmargus*, but largely due to the extent of compressions of the egg, for it has gone farther in those eggs which are compressed the most. The entoblast nuclei migrate to the surface of the yolk and form the enteron or mid gut, as described by Pedaschenko.

V. POLYSPERMY.

In *Læmargus muricatus* I have found many eggs into which a number of spermatozoa had entered. In one case the whole egg string was of such eggs; in the other cases only a few such eggs were found in a string. The "development" of these eggs falls under three classes:

1. The ♀ pronucleus and the ♂ pronuclei fuse to form one nucleus in the center of the egg which does not develop further.
2. In the center of the egg a multipolar spindle is formed usually of three principal poles and one minor pole. The resulting division in all observed cases cleaves the egg into three subequal cells, in each of which a bipolar spindle with a very large number of chromosomes is formed. Further development is very irregular.
3. A bipolar spindle with an immense number of chromosomes is formed in the center of the egg. Apart from the number of chromosomes the cleavage approaches the normal type, especially up to the 4 cell stage after which it diverges more and more from the normal type. I am led to believe by certain eggs that show an intermediate stage between a multipolar and a bipolar spindle, that the bipolar spindles in the first cleavage of these eggs are formed out of multipolar spindles.

As all of these eggs were already mounted (by Professor Rynearson) I was not able to observe whether the axes of these polyspermous eggs that approached the normal type in development were the same as in normal eggs. I have not observed whether maturation takes place in polyspermous eggs — the cleavage spindles are very different from normal cleavage spindles, and are very similar to normal maturation spindles. This may be due to a tendency to throw out the excess of chromatin, and in some cases I have found a mass of very small cells extruded from the egg, not always, however, in the position in which polar bodies normally form. There are often many asters in the egg unconnected with chromosomes, and this may account for rounded masses of yolk that are sometimes cut off from the egg.

VII. RELATION OF PRESSURE, ETC., TO THE TYPE OF CLEAVAGE.

When the eggs are released from the oviduct in sea water, they begin slowly to round up and separate one from another. The eggs adhere together so strongly that their tendency to assume a spherical form is greatly impeded, and it always takes several hours for them to round up. The majority of the eggs liberated begin to disintegrate before they proceed very far toward becoming isodiametrical. This is due to their very low surface tension, their cohesion being less than their adhesion for the surface film of sea water or for glass. This is shown by the fact that the eggs tend to stick to the bottom of the glass dish containing the sea water, and when the dish is tilted so that some eggs come in contact with the surface of the water, they quickly spread out over that surface. All these experiments support the direct observation that the oöcyte is surrounded by no other membrane than its surface film.

If eggs are left standing in sea water more than two to four hours their surfaces begin to disintegrate. This is probably caused by partial solution in sea water. The nuclei remain intact after a great deal of the egg has disintegrated. If the eggs are placed in hypotonic solutions they swell, if in hypertonic solutions, they shrink, without any other change that can be observed. I tried solutions of magnesium chloride, ether, and sodium hydroxide, of varying strengths in sea water containing eggs alone or eggs and sperm but could neither induce parthenogenesis nor fertilization. The spermatozoa are very similar to those of cirripedia, being thread-like and each containing a homogeneous thread of chromatin running the entire length. The sperm of many crustacea are non-motile when examined in sea water or serum, but some of them have been observed to perform movements in the female genital ducts. Cano ('93) saw decapod spermatozoa move lively in the Rec. seminis. It is therefore probable that I did not find the proper stimulus to cause fertilization in sea water. Immediately after fertilization and passage into the egg strings the egg secretes a chitinous chorion that resists all attempts at freeing the eggs so that they will round up, without mutilating them, so I had to resort to looking for eggs that by accident were not flattened in the usual manner. In the Dichelestid the egg at each end of the string is hemispherical in shape, due to the fact that it is pressed on only one side (Fig. 36). In the proximal egg the ventral side is rounded and in the distal egg the dorsal side is rounded. The first protoplasmic cell (*ab*) is cut off at one edge of the hemisphere. The second cleavage results in the formation of three protoplasmic cells (*a*, *b*, *c*) whose centers form the apices of a triangle on the spherical surface at its edge (Fig. 36, *A* and *B*). We should assume that this arrangement is nearer the ancestral type, which was probably a sphere, and that the first three protoplasmic cells being in the equatorial plane (Fig. 27) is due to the pressure. Fig. 36, *A* and *B*, shows a similar arrangement of cells to the same stage in the cleavage of *Lepas* as figured by Biglow, save that in the dichelestid the yolk is much greater in amount and one side of the egg is flat. In both cases *d* (the yolk cell) extends under *a*, *b*, and *c* but in the dichelestid the yolk cell is so large as to push *c* over *b* (in the distal egg).

This altered arrangement of the protoplasmic cells does not seem to affect the normal development of the embryo. The ectoderm grows over the yolk in the usual manner, except that it is stretched more on the rounded side of the egg (Fig. 36, *C*). The four entoderm cells are thicker, and in the distal egg of more volume, than normally and after the entoderm forms a syncytium the nuclei have not exactly their normal arrangement, but when the ensuing nauplius escapes from the egg membrane everything is apparently restored to its normal relation, save that a nauplius developing from a distal egg is larger.

This is contrary to the idea of Schimkewitz, who attributes many abnormalities in parasitic copepod embryos to slight differences in pressure in the egg string; but the eggs he studied had less yolk than those considered in this paper. Differences in pressure in the dichelestid egg result principally in differences in form of the yolk mass. This yolk mass does not, save to very small extent, enter as such into the composition of cells, but is dissolved and used as food by the cells. The protoplasmic cells always being on the surface of the yolk, their relation to the food supply remains unchanged.

Experiments on the effect of unequal compression on cleavage have been made on *Ascaris* eggs by Auerbach ('74); on amphibian eggs by Pflüger ('84), Roux ('85, '93), Born ('93, '94), O. Hertwig ('93); on echinoderm eggs by Driesch ('92, '93, '95), Morgan ('93), Ziegler ('94); on ctenophore eggs by Ziegler ('94); and on *Nereis* eggs by Wilson ('95). These experiments show that if the egg is pressed more on certain sides than on others, as when it is pressed between two plates of glass and forced to assume a flattened shape, that the direction of the cleavage spindles (and consequently cleavage furrows) will be affected. Hertwig formulated the law that the spindle lies in the longest axis of the protoplasmic mass of the cell. This rule probably applies in the majority of cases, but there may be some exceptions, and there are evidently other and unknown factors which enter into the polar differentiation of the cell. Bigelow found in *Lepas* ('02) that the polarity of the egg was not affected by the oval form of the rigid chorion. The principal axis of the egg coincided with the long axis when the chorion was secreted and during the prophase of the first cleavage spindle the egg rotated through a right angle so that the first cleavage spindle was made to coincide with the long axis of the egg determined by the form of the chorion, and the principal or primary axis was perpendicular to it.

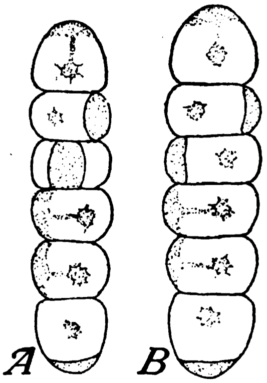


FIG. 4. Egg strings of *Endactylina nigra* Wilson. The protoplasm is stippled and the yolk white, the distinctness between the two being accentuated. *A*, Living egg string during first cleavage. *B*. The same compressed between the slide and cover glass.

I found a similar condition in *Eudactylina nigra* Wilson. If the egg string of this copepod be placed in sea water under the microscope during the first cleavage stage, the majority of the eggs will have their spindle axes, or in case the division is complete, common cell axis, nearly in the same plane. Often, however, some of these axes are considerably inclined to this plane as is shown in Fig. 4, *A*. If the egg string be pressed between slide and cover glass the above axes will rotate sufficiently to bring them all in a plane midway between slide and cover glass and therefore in the same plane (Fig. 4, *B*). Thus the first cleavage obeys Hertwig's rule whether the compression be applied during or after the metaphase and possibly during the ensuing resting stage.

Hertwig says that if frog eggs are thus compressed normal embryos will develop, although a totally different distribution of nuclei results.

Born found that if a frog's egg were inverted before formation of first cleavage spindle the relative density of protoplasm and yolk would cause streaming movements in the egg, the protoplasm and nucleus rising through the yolk to the upper pole. But it is probable that these streaming movements would be hindered by the astral rays after formation of the spindle.

In the parasitic copepods the direction of many of the spindles is influenced by the pressure, and Hertwig's law applies in most cases. But the peculiar form of cleavage seems well adapted to variations in pressure. The blastoderm lying on the yolk may be compared to a rubber bag divided by lines into polygonal areas. The bag may be pressed into various shapes without altering the mutual relation of adjacent polygons. The only cell whose form is changed very much by pressure is the yolk cell.

I have said that the distal egg (the one at the free end of the egg string) is larger than the others. This is due to the fact that in the oviduct it presented more surface for absorption of nutriment through the wall of the oviduct. While the other eggs present only a thin edge toward the source of food, this last egg of the series presents this edge and one whole flat side in addition. Usually it does not remain flat, but becomes more or less hemispherical on the free side while still in the oviduct.

The cytoplasm of many cells is formed in large part from the substances that escape from the nucleus at the first maturation and early cleavage divisions. Dr. Conklin traced a similar process in gasteropods and in the living eggs of ascidians, and it may be a general phenomenon. In other words, a quantity of chromatin is dissolved and escapes into the cytoplasm in many and perhaps all cell divisions.

In Paramœcium the macronucleus and a large part of the substance of the micronucleus escapes into the cytoplasm at each copulation and may constitute necessary ingredients of the cytoplasm, as copulation is necessary to long continued existence of Paramœcia. It is probably chiefly thus that the heritable qualities residing in the chromosomes are conveyed to the cytoplasm. I do not mean to say that this is the only way the nucleus affects the cytoplasm, for with a few exceptions (*i. e.*, red blood corpuscles of higher animals) cytoplasm not containing a nucleus soon dies, but if the heritable qualities are stored up in the chromatin, part of this chromatin bearing these qualities could be transferred to the cytoplasm more easily during the absence of a nuclear membrane.

After the close of the fifth cleavage (32 cells) the embryo is composed of three types of cells that differ visibly.

1. The primary germ cell.
2. The primary entoblast cell.
3. Thirty cells of the blastoderm all similar in appearance.

The primary germ cell when first separated from the entoderm looks like the other cells of the blastoderm, but during the rest grows larger than its neighbors and is delayed in mitosis. In this character of delayed mitosis it resembles its sister cell (primary entoderm cell). There is nothing characteristic of its position that could cause it to become different from its neighbors, so we must ascribe this difference to the difference in the substances entering into it which in turn may be caused by unequal cleavage.

VII. SUMMARY.

1. My observations on the cell lineage agree in general with those of Pedaschenko (who worked it out to the 16 cell stage) save in regard to the orientation. Pedaschenko used no means to distinguish between the two flat sides of the egg and was mistaken in regard to the location of the animal pole, as I have shown (p. 50). At the fifth cleavage the yolk cell buds off the last protoplasmic cell, which is the primary germ cell. After extrusion of the germ cell (32 cell stage) the yolk cell is purely entoblastic. The segregation of the entoblast takes place one generation later than in *Lepas* (Biglow '02), and the segregation of the germ cells one generation later than in *Cyclops* (Haecker). The delay in the segregation of these two elements is probably due to the large amount of yolk present and the compressed condition of the egg, which cause delay in gastrula-

tion (epibole). It is probable that all the mesoderm arises from cells turned under the lip of the blastopore.

2. The entrance of supernumerary spermatozoa into the egg so greatly disturbs the process of development that the latter is either prevented or so distorted that it never progresses very far, and then in an abnormal manner.

3. The compressed condition of the egg affects the cleavage: (1) by altering the arrangement of the protoplasmic cells, (2) by necessitating increased length of the spindles in the yolk cells, (3) by preventing cleavage of the yolk, and (4) by increasing the surface of the egg and retarding gastrulation (epibole). But it is very improbable that slight alterations in the amount and direction of compression have as great an influence on development as supposed by Schimkewitz. I found nauplii which were apparently normal (save perhaps in size) hatching from hemispherical eggs. As the nauplius hatches it immediately rounds up, and assumes the same form whether it arise from a hemispherical or from a very flat egg.

VIII. EXPLANATION OF PLATES.

ABBREVIATIONS.

an^1 = First antenna.
an^2 = Second antenna.
b = Blastopore.
e = Entoblast cell.
en = Entoblast nucleus.
ec = Ectoderm cell.
f = Deeply staining protoplasm.
g = Germ cell.
m = Muscle cell.
m^2 = Mesoblast of first antenna.
m^3 = Mesoblast of second antenna.
m^4 = Mesoblast of mandible.
m^{5-10} = Mesoblast of post nauplius appendages.
md = Mandible.
mp = Postnauplius mesoblast.
n^1 = Procerebrum.
n^2 = Neuromere of first antenna.
n^3 = Neuromere of second antenna.
n^4 = Neuromere of mandible.
n^{5-10} = Neuromeres of post nauplius segments.
o = Rudiment of mouth.
o^1 = Rudiment of lateral eye.
om = Rudiment of median eye.
x = Darkly staining cell in yolk.

IX. BIBLIOGRAPHY (OF BOTH PARTS).

Auerbach, L.

'74 Organologische Studien. Breslau, 1874.

Berg, Walt.

'05 Weitere Beiträge zur Theorie der histologischen Fixation Versuche an Nucleinsauren Protamin. Arch. Anat., 65.

Berthold, G.

'86 Studien über Protoplasmamechanick. Leipzig, 1886 (Felix).

Bigelow, M. A.

'02 The Early Development of Lepas. A Study of Cell Lineage and Germ Layers. Bull. Museum Comp. Zoo. Harvard College, XL., No. 2.

Born, G.

'93 Ueber Druckversuche an Froscheiren. Anat. Anz. 8.

'94 Nene Compressionsversuche an Froscheiren. Jahr. Schles. Ges. f. Vaterl. Cultur. Zoo. Bot. Sect., 1894.

Boveri, Th.

'92 Die Entstehung des Gegensatzes zwischen den Geschlechtzellen und den somatischen Zellen bei Ascaris megalocephala. Sitzungsber, Ges. für Morph. Physiol. München, Bd. 8.

Brauer, A.

'92 Ueber das Ei von Branchipus grubei von der Bildung bis zur Ablage. Anhandl. Akad. Wiss. Berlin, 1892.

Bütschli, O.

'91 Ueber die sogenannten Centralkörper. Verh. d. Nat. Med. Ver. zu Heidele berg, IV.

'92 Untersuchungen über mikroscopische Schäume und das Protoplasma. Leipsig, 1892.

'98 "Untersuchungen über Structuren, inbesondere, über S. nichtzelliges Erzen. guisse des Organismus und über ihre Beziehungen zu Structuren, welche ausserhalb des Organismus entstehen. Leipzig, 1898.

'00 Bemerkungen über Plasmastromungen bei der Zelltheilung. Arch. Entwichlungmechanic, X.

Cano, G.

'93 "Svilluppo dei Dromidei. Atti R. Acad. Sc. Fis. et Mat., Vol. 6, Ser. 2, No. 2, Napoli, 1893.

Canu, E.

'92 Les Copepods du Boulonnais Trav. Lab. z. Wimereux-Ambleteuse Tome 6. Lille.

Carnoy, J. B.

'85 La Cytodierese chez les Arthropodes. La Cellule, Vol. I.

Casteel, C. D.

'04 The Cell Lineage and Early Development of Fiona marina, a Nudibranchiate Mollusc. Proc. Acad. Nat. Sc. Phila., '04.

Conklin, E. G.

'97 The Embryology of Crepidula, a Contribution to the Cell Lineage and Early Development of some Marine Gasteropods. Jour. Morph., 13.

'02 Karyokinesis and Cytokinesis in the Maturation, Fertilization and Cleavage of Crepidula and other Gasteropoda. Jour. Acad. Nat. Sc. Phila., XII. (2d ser.).

'05 The Organization and Cell Lineage of the Ascidian Egg. Jour. Acad. Sc-Phila., XIII., pt. I.

Crampton, H. E.

'99 Studies upon the Early History of the Ascidian Egg. Jour. Morph., XV. Supplement.

Driesch, H.

'92 Entwicklungsmech. Studien, IV. Zeit. Wiss. Zoo., 55.

'93 Zur Verlagerung der Blastomeren des Echinideneis. Anat. Anz., 8.

'05 Die Entwicklungsphysiologie von 1902 bis 1905. Ergenbuisse der Anat. u. Entw., 14, '04.

Eigenmann, C. H.

'91 On the Precocious Segregation of the Sex Cells in Micrometrus aggregatus Gibbons. Jour. Morph. Boston, Vol. 5.

Fischel, A.

'99 Ueber vitale Färbung von Echinoderm eiern wahrend ihrer Entwickelung. Anat. Hefte, 1 Abt., 11.

Fischer, A.

'99 Fixierung, Färbung und Bau des Protoplasmas, Jena.

Foot, K.

'96 Yolk Nucleus and Polar Rings. Jour. Morph., XII.

Fürth, O. von.

'03 Vergleichende Chemische Physiologie der niederen Tiere. Jena (Gustav Fisher), 1903.

Gerstaecker, A.

'66 Copepoda. Bronn's "Klassen und Ordnungen des Thierreichs" V., pt. 2.

Giard.

'87 Sur un Copepod parasite de l'Amphiura squamata. Comp. Rend., Tome 104, pp. 1189–92.

Giesbrecht, W.

'82 Beiträge zur Kenntniss einiger Notodelphyiden. Mit. Zoo. St. Neapel, 3 Bd.

Gilson, S.

'86 Etude comparee de la spermatogenese chez les Arthropodes. La Cellule, Tome 1 and 2, p. 140.

Grobben, C.

'79 Die Entwickelungsgeschichte der Moina rectirostris. Arb. Zoo. Ins. Wien, 1 Bd., 2 Hft.

'81 Die Entwichelungsgeschichte von Cetochilus septentrionalis. Arb.Zoo. Ins. Wien, 3 Bd.

Gruber, Aug.

'79 Beiträge zur Kenntniss der Generationsorgane der freilebenden Copepoden. Zeit. Wiss. Zool., 32 Bd., p. 407.

Haecker, V.

'91 Die Richtungskorperbildung bei Cyclops und Canthocamptus. Preliminary. Ber. Nat. Ges. Freiburg, 6. Bd., Bio. Cent., 11.

'92 Die Korntheilungsvorgänge bei der Mesoderm und Entodermbildung von Cyclops. Arch. Mikr. Anat., 39 Bd.

'92, a Die Eibildung bei Cyclops und Canthocamptus. Z. Jahrb. Morph., Abt., 5 Bd., p. 211.

'93 Das Keimbläschen, seine Elemente und Lagerveranderungen 1. Uber die biologische Bedeutung des Keimblaschenstadiums und über die Bildung der Vierergruppen. Arch. Mikr. Anat., 41 Bd., p. 452.

'94 Die Entwicklung der Wintereier der Daphniden. Ber. naturf. Gesell. Freiburg, 8 Bd.

'94, a Ueber generative und embryonale Mitosen, sowie über pathologische Kerntheilungsbilder. Arch. Mikr. Anat., 43 Bd.

'95 Ueber die Selbstandigkeit der väterlichen und mütterlichen Kernbestandtheile während der Embryonalentwickelung von Cyclops. Arch. Mikr. Anat., 46 Bd., p. 579.

'95, a Die Vorstadien der Eireifung. Arch. Mikr. Anat., 45 Bd., p. 200.

'97 Die Keimbahn von Cyclops. Arch. Mikr. Anat., 49 Bd., p. 35.

'02 Ueber das Schicksal der elterlichen und groselterlichen Kernantheile. Morphologische Beiträge zum Ausbau der Vererbungslehre. Jena. Zeit. Natur., 37 Bd., p. 297.

Hansen, H. J.

'00 Danmarks Stilling og Tilstand. 2. Det. kongelige Danske Videnskabernes Gelskab. Kjöbenhavn, 214 pp., 10 figs.

Heath, H.

'99 The Development of Ischnochiton. Zoo. Jahr., XII.

Heider, K.

'79 Die Gattung Lernanthropus. Arb. Zoo. Ins. Wien, 2 Bd., 3 Hft.

'92 Crustacea. Lehrbuch der vergleichenden Entwickelungsgeschichte der wirbellosen Thiere. Jena, 1892.

Hermann, S.

'83 Sur la Spermatogenese des Crustace Podopthalmes, specialment des Decapodes. Compt. Rend., Tome 97, p. 958.

Hertwig, O.

'93 Ueber den Werth der ersten Furchungszellen für die Organbildung des Embryo. Arch. f. Mic. Anat., 42.

Ishikawa, A.

'92 Studies in Reproductive elements. 1. Spermatogenesis, Oögenesis and Fertilization in Diaptomus sp. Jour. Coll. Sc. Japan, Vol. 5.

Jennings, H. S.

'96 The Early Development of Asplanchna herrickii. Bull. Mus. Comp. Zoo., XXX., 1.

'04 Physical Imitations of the activities of Amœba. Amer. Nat., XXXVIII.

Kofoid, C. A.

'94 On some Laws of Cleavage in Limax. (Preliminary.) Proc. Am. Acad. Arts and Sc., V., 29.

Korschelдt & Heider.

'02–'03 Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere. Allegemeiner Theil., Jena.

Kröyer, H.

'37–8 Om Snyltekrebsene, isaer med. Hensyn til Dansk. Faune. Naturhistorisk Tidsskrift I. and II.

'63 Bidrag til Kundskab om Snyltekrebsene. Naturhistorisk Tidsskrift, Tredie Raekke. Andet Bind., pp. 75–426.

Labbé, A.

'04 Sur la formation des tetrades et les divisions maturatives dans le testicule du Homard. C. R. Acad. Sc., Paris, Tome 138, p. 96.

Lee, A. B.
'05 The Microtomist's Vade-Mecum. 6th Ed., Phila., '05.
Lerat, Paul.
'02 La premiere cinese de maturation dans l'Ovogenese et la spermatogenese du Cyclops strenuus. Noté preliminaire. Anat. Anz., 21 Bd., p. 407.
Lillie, F. R.
'95 The Embryology of the Unionidæ. A Study in Cell Lineage. Jour. Morph., 10.
'01 The Organization of the Egg of Unio. Jour. Morph., XVII.
Mathews, A. P.
'98 A Contribution to the Chemistry of Cytological Staining. Am. Jour. of Phys., I., No. 4.
Mann, G.
'06 Chemistry of the Pretoids.
Mark, E. L.
'81 Maturation, Fecundation and Segmentation of Limax Campestris. Bull. Mus. Comp. Zoo. Harvard College, Vol. VI., No. 12.
Mead, A. D.
'97 The Early Development of Marine Annelids. Jour. Morph., XIII.
Meyer, E.
'01 Studien über den Körperbau der Anneliden. V. Das Mesoderm der Ringelwürmer. Mitt. Zoo. Sta. Neapel, 14 Bd.
Morgan, T. H.
'93 Experimental Studies on Echinoderm Eggs. Anat. Anz., 9.
'96 The Production of Artificial Astrospheres. Arch. Entwickelungsmechanik, III.
Montgomery, T. H.
'98 The Spermatogenesis of Pentatoma. Zoo. Jahrb., XII.
'99 Comparative Cytological Studies with special reference to the Morphology of the Nucleolus. Jour. Morph., XV.
'00 The Spermatogenesis of Peripatus. Zoo. Jahrb., XIV.
Nelson, J. N.
'04 The Early Development of Dinophilus. Pro. Acad. Natl. Sc. Phila., Oct., '04.
Nusbaum, and Schreiber, W.
'98 Beiträge zur Kenntnis der sogen. Rückenorgane der Crustaceenembryonen. Bio. Centralbl., 18 Bd., p. 736.
Norman, W. W.
'96 Segmentation of the Nucleus without Segmentation of the Protoplasm. Archiv. f. Entwickelungsmech., III.
Pauli, W.
'02 Allgemeine Physiko-Chemie der Zellen und Gewebe. Ergebnisse der Physiologie Wiesbaden, 1 Jahr., I. Abt., Bio-Chemie.
Pedaschenko, D.
'93 Sur la segmentation de l'oeuf et la formation des feuillets embryonnaires chez la Lernae branchialis. (Preliminary) Revue Sc. N. Peterbourg Tome 37.
'99 Embryonalentwickelung und Metamorphose von Lernae branchialis, L. Trav. Soc. Nat. Petersbourg, Vol. 26.

Pflüger, E.

'84 Ueber die Einwirkung der Schwerkraft und anderer Bedingungen auf die Richtung der Zelltheilung. Arch. Ges. Physiol., 34.

Rath, O. Vom.

'92 Zur Kenntniss der Spermatogenese von Gryllotalpa vulgaris. Arch. Miks. Anat., 40 Bd., p. 102

Reed, Margaret.

'05 Formation of the interior cells in the segmentation of the Frog's egg. Biol. Bull., Feb., 1905, Vol. 8, No. 3.

Reinke, F.

'00 Ueber den Mitotischen Druck. Arch. f. Entwickelungsmech., IX.

Rhumbler, L.

'96 Versuch einer mechanischen Erklarung der indirekten Zell und Kerntheilung. I. Die Cytokinese. Arch. Entwickelungsmechanik Organ., 3.

'05 Zur Theorie der Oberflächenkräfte der Amöben. Zeit. Wiss. Zoo., 83.

Robert, A.

'03 Recherches sur le development des Troques. Arch. Zoo. Exper. Serie X.

Roux.

'85 Beitr. z. Entw-Mech. d. Embryo. III. Bresl. Aerzth. Zeitschr., 1885, Ges. Abh., II. Bd., No. 20.

'93 Ueber Mosaikarbeit und neuere Entwicklungshypothesen. Anat. Heftes, 1893, Ges. Abh., II. Bd., No. 27.

Ruckert, J.

'94 Zur Ereifung bei Copepoden. Anat. Hefte, 1 Abth., 4 Bd., p. 261.
Ueber das Selbständigbleiben der väterlichen und mütterlichen Kernsubstanz während des ersten Entwickelung des befruchteten Cyclops Eis. Arch. Mikr. Anat., 45 Bd., p. 339.

'95, a Zur Kenntnis des Befruchtungsvorganges. Sitz. Ber. Akad. München, 25 Bd.

'95, b Zur Befruchtung von Cyclops strenuus. Anat. Anz., 10 Bd., p. 708.

'96 Nochmals zur Reductionsfrage. Arch. Mikr. Anat., 47 Bd., p. 386.

Schimkewitz, W.

'96 Studien über parasitische Copepoden. Zeit. Wiss. Zoo., 2, 61 Bd., p. 339.

'99 Einige Worte über die Entwickelung der parasitischen Copepoden. Z. Anzeiger, 22 Bd., p. 14.

Schläpfer.

'05 Eine physikalische Erklarung der acromatischen Spindelfigure, etc. Arch. Entw. Mech., 19, 1905.

Steuer, A.

'03 Mytilicola intestinalis n. g., n. sp. Arb. Z. Ins. Wien., 15 Bd.

Treadwell, A. L.

'01 The Cytogeny of Podarke obscura. Jour. Morph., 17, No. 3.

Urbanowitz, F.

'85 Beiträge zur Entwickelungsgeschichte der Copepoden. Kosmos Lemberg 10 Jahrg. & Berichten Warsch. Universität, 1885, and Arch. Slav. Biol., Tome 1, p. 663 (Review, '86).

Wagner, J.

'93 Einige Betrachtungen über die Bildung, der Keimblätter, der Dotterzellen und der Embryonalhüllen bei Arthropoden. Bio Centralbl., 14 Bd., p. 361.

Weismann & Ischikawa.

'88 Ueber die Bildung der Richtungskorper bei thierischen Eiern. Ber. Nat. Ges. Freibourg, 3 Bd.

'88, a Weitere Untersuchungen zum Zahlengesetz der Richtungskörper. Z. Jahrb. Morph. Abth., 3 Bd.

Wheeler, Wm. M.

'93 A contribution to Insect Embryology. Jour. Morph., Vol. 8.

'97 The Maturation, Fecundation and early Cleavage in Myzostoma. Arch. de Biol., XV.

Wierezjski, A.

'05 Embryologie von Physa fontinalis. Zeit. Wiss. Zoo., 83.

Wilson, C. B.

'05 New Species of Parasitic Copepods from the Massachusetts Coast. Pro. Biological Soc. Washington.

05, a North American Parasitic Copepods belonging to the Family Caligidæ. Pt. I. The Caliginæ. Pro. U. S. Nat. Museum, XXVIII., pp. 479–672, pl. V.—XXIX.

Wilson, E. B.

'92 The Cell Lineage of Nereis. Jour. Morph., VI.

'95 On Cleavage and Mosaic-work. Appendix to Crampton. Arch. f. Entw Mech., 3.

Wright, R. R.

'83 Notes on American Parasitic Copepoda, I. Proc. Canad. Ins. (2), Vol. I., p. 243.

Ziegler, H. E.

'94 Ueber Furchung unter Pressung. Verh. Anat. Gessellsch. 8 Vers. Strassburg, 1894 (Anat. Anz. Supple.).

'98 Ueber den derzeitigen Stand der Cölomfrage. Verh. der deutsch. Zoo. Ges., VIII.

PLATE II.

(*Figs. 19–24, Læmargus muricatus Kröyer.*)

FIG. 19. First cleavage spindle, prophase.

FIG. 20. First cleavage spindle, metaphase.

FIG. 21. Early prophase of second cleavage. To the right, below, is a highly magnified section of the centrosomes in which the centrodesmus and nucleus of the yolk cell are shown.

FIG. 22. A little later prophase of the same. In the protoplasmic cell the nuclear membrane has begun to dissolve.

FIG. 23. Later prophase showing the elongation of the nucleus of the yolk cell. Viewed from the animal pole.

FIG. 24. Late prophase (the protoplasmic cell is in the metaphase) viewed from the vegetal pole.

Figs. 25–30, The Dichelestid. All eggs viewed from vegetal pole.)

FIG. 25. Anaphase of the first cleavage (fixation poor?).

FIG. 26. Two cell stage. The protoplasmic cell is in the anaphase of the second cleavage.

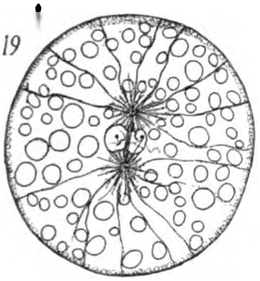
FIG. 27. Anaphase of the second cleavage viewed from the vegetal pole (the, protoplasmic cell is in the telophase).

FIG. 28. Prophase of third cleavage.

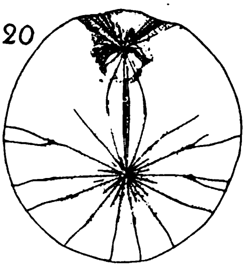
FIG. 29. Late prophase of fourth cleavage $a^{4.2}$, $b^{4.2}$ and $c^{4.2}$ are almost completely hidden by cells lying over them.

FIG. 30. Spindle in the yolk cell, metaphase of fourth cleavage, from two consecutive sections and magnified more highly than Fig. 29. Stained with safranin-gentian-violet. The distinctness of the "centrosomes" is exaggerated. The dark granules on the astral rays of the sphere to the right are lumps of hyaloplasm. The delicate network of hyaloplasm between the yolk spherules is represented by dotted lines but the yolk itself is not shown.

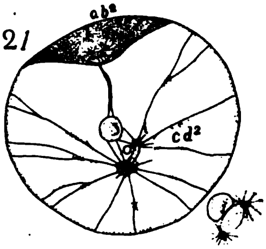
19



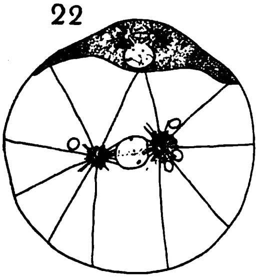
20



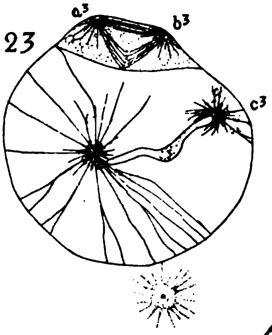
21



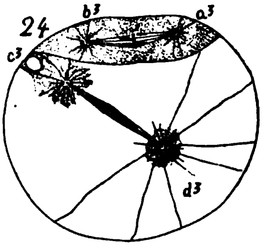
22



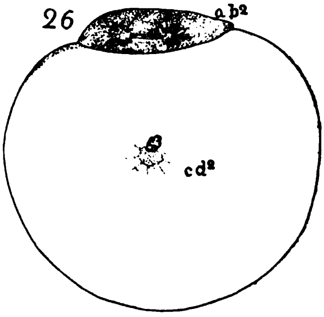
23



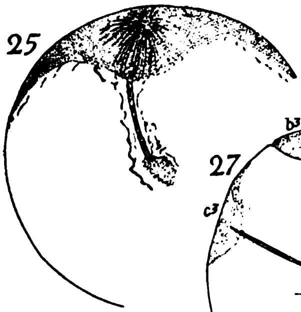
24



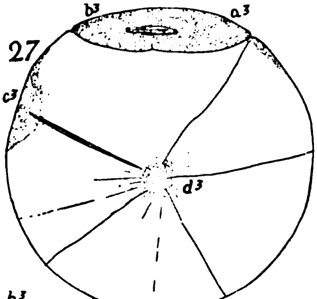
26



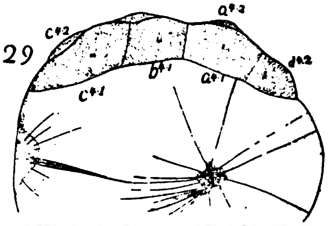
25



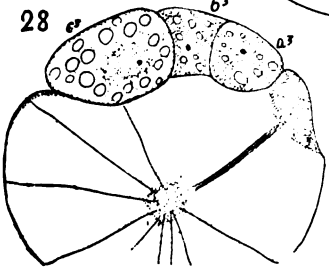
27



29



28



30

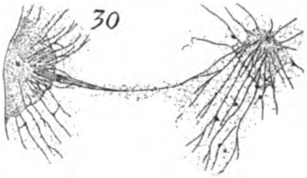


PLATE III.

(In Figs. 31, 32 and 33 the derivatives of *a*, *b*, *c* and *d* are separated by heavy lines.)

FIG. 31. Sixteen cell stage. The cells of the animal-pole-side are shown by dotted lines. $a^{5.1}$, $b^{5.1}$ and $b^{5.2}$ are in the metaphase of the fifth cleavage.

FIG. 32. Thirty two cell stage. The cells of the animal-pole-side are shown by spaced lines.

FIG. 33. Sixty two cell stage. The primary entoblast, $d^{6.1}$, and the primary germ cell, $d^{6.2}$, have not begun the sixth cleavage while $d^{7.10}$, $d^{7.11}$ and $d^{7.12}$ are in the metaphase or anaphase of the seventh.

[*Figs. 34–36, The Dichelestid. Both embryos seen from ventral (vegetal) side.*]

FIG. 34. A later stage than the one shown in Fig. 36, *B*, Plate VI. The primary entoblast cell is dividing. The primary germ cell (*g*) has grown to large size and the blastoderm is beginning to grow over it. At the sides of the figure some mesoblast cells have been turned under the rim of the blastopore (m^{2+3}).

FIG. 35. A later stage than Fig. 34. The entoblast is in the telophase of the second division. The yolk is cut through completely by both divisions of the entoblast. The primary germ cell has divided (*g*) and the blastoderm has grown over it.

FIG. 36. Hemispherical eggs from the ends of egg strings.

A. Dorsal view.

B. Anterior view.

C. Lateral view of stage in which the blastoderm (stippled) has covered half the yolk; the entoblast nuclei are stippled heavily.

(*Figs. 49–56, Pandarus sinuatus.*)

FIG. 49. Sagittal section of gastrula just before the closure of the blastopore (*b*). *p* = first polar body. *mp* = a cell of the post nauplius mesoblast. *x* is taken from another section of the same series and shows a small cell budded off into the yolk from an ectoderm cell. *g* is from one of the same series of sections near the median line, and represents a germ cell in its relation to the ectoderm.

FIG. 50. Part of a median cross-section of an embryo of stage *C* (Fig. 44). The section passes through two germ cells (*g*) a muscle cell (*m*) and two entoblast nuclei. The thickened portion of the ectoderm on the ventral side is the ganglionic rudiment of the second antenna.

FIG. 51. The nauplius just hatched. The ventral aspect is shown in the left, the dorsal in the right half of the figure. The median eye is seen at *om*, and the stomodæum at *o*. The rudiments of the post nauplius ganglia (n^{5-10}) and appendages (m^{5-10}) are clearly differentiated. The entoblasts (*en*) are still scattered through the yolk.

FIG. 52. Section of one of the four germ cells of the nauplius showing the sixteen chromosomes.

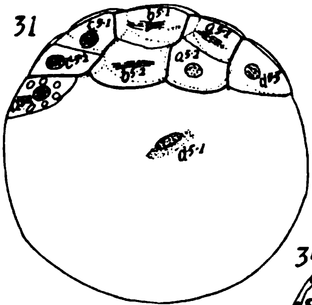
FIG. 53. Enlarged view of section of divided cell from rim of blastopore in Fig. 40. All the chromosomes are not included in the section.

FIG. 54. Prophase from same region.

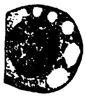
FIG. 55. Telophase.

FIG. 56. Cross-section through germ cells, ventral ganglia, and ectoderm of nauplius twenty-four hours after hatching.

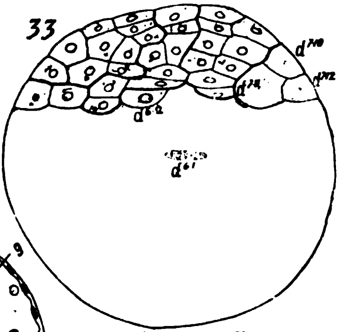
31



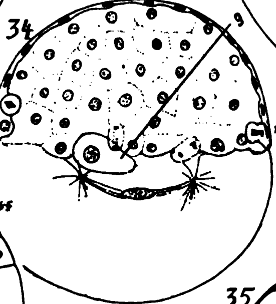
52



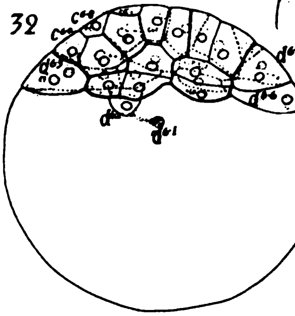
33



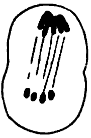
34



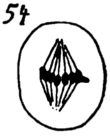
32



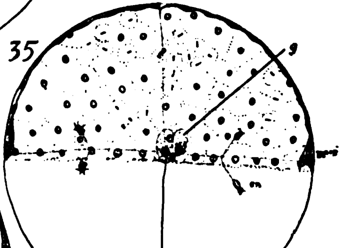
53



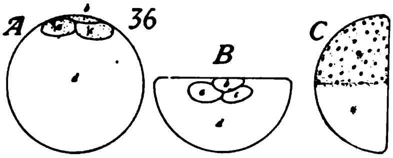
54



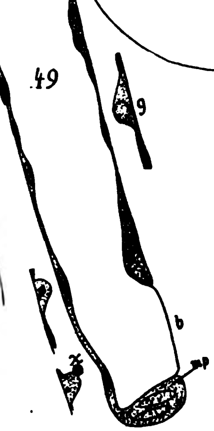
35



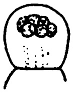
36



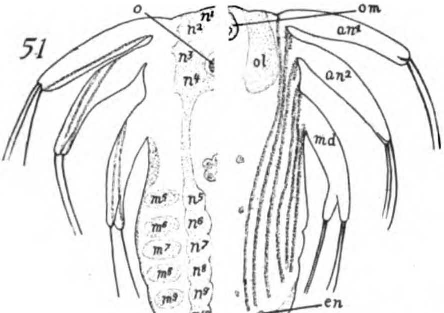
49



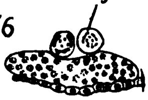
55



51



56



50

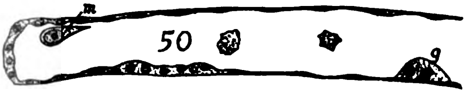


PLATE IV.

(*Pandarus sinuatus Verrill. Figs. 37–39, viewed from the vegetal pole.*)

FIG. 37. Shows the metaphase of the first cleavage of the primary ectoblast cell. To the right below is a more highly magnified view of the spindle of the same, in the prophase. g = primary germ cell.

FIG. 38. Shows the prophase of the second cleavage of the entoblast. The blastoderm is growing over the primary germ cell (g). Some mesoblast cells have been turned under the rim of the blastopore at the sides of the figure.

FIG. 39. About the same stage as Fig. 38, but the entoblast has not begun its second cleavage and the primary germ cell (g) is in the metaphase of division.

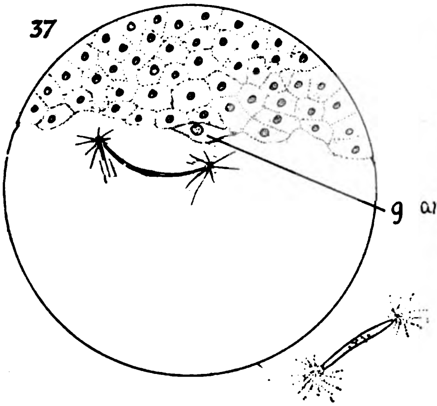
[In Figs. 40–42 half of the dorsal (animal) side of the egg is shown to the left and half of the ventral (vegetal) side to the right.]

FIG. 40. Just before closure of the blastopore (b). X = small cell budding into the yolk. An = mesoblastic rudiment of the first antenna. An^2 = mesoblastic rudiment of second antenna. Md = mesoblastic rudiment of the mandible. The germ cells are shown (stippled) beneath the ectoderm — they have separated from one another.

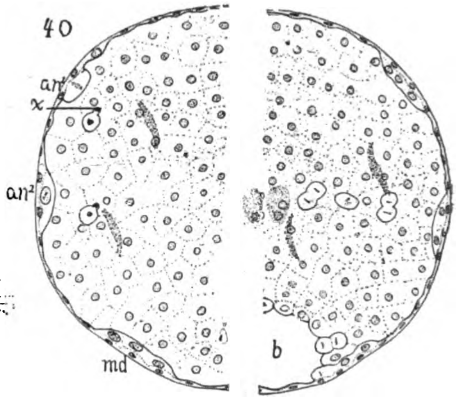
FIG. 41. Stage A. Just after closure of the blastopore. The dorsal and ventral ectoderm is omitted save in the anterior and posterior portions. m, m = mesoblast (muscle?) cells, just beneath the ectoderm. The germ cells have divided.

FIG. 42. Stage A a little later than 41. The eight stippled rods are the spindles of the entoblast. The other stippled areas are mesoblast.

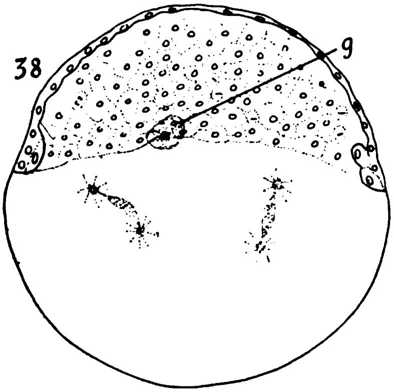
37



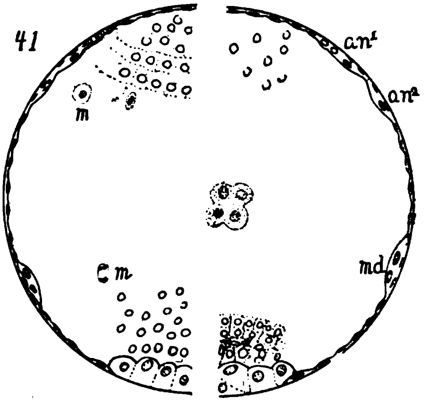
40



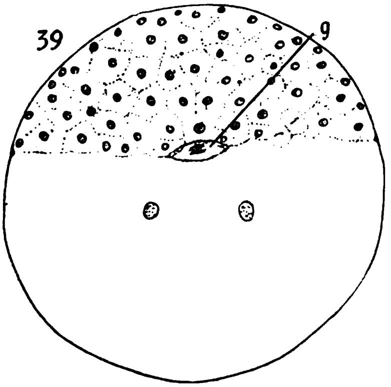
38



41



39



42

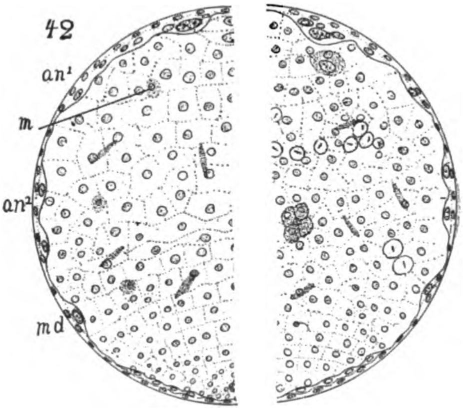


PLATE V.

(*Pandarus sinuatus Verrill.*)

(One half of each figure shows the dorsal, the other half the ventral aspect. In Figs. 43 and 45 the ventral aspect is to the right and in the remaining figures to the left.)

FIG. 43. Stage *B*. The rudiments of the ganglia, n^{1-4}, are shown. The strippled rods are spindles of entoblast nuclei.

FIG. 44. Stage *C*. At *x* are minute (mesoblast?) cells in the yolk. Some muscles in the right side of the figure are beginning to elongate. Over the ganglion of the second antenna is a cluster of mesoblast cells of unknown history.

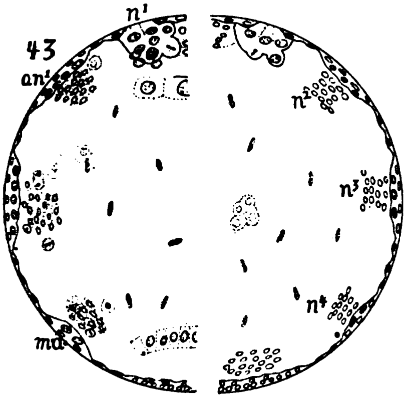
FIG. 45. Stage *D*. The appendages are beginning to bud out. The ganglia have become connected by thickened ectoderm (outlined by a dotted line). The muscle-cells are beginning to elongate radially, and above them the ectoderm cells are elongated in the opposite direction (some of them are represented by dotted outlines, *ec*). The entoblast is not represented, but a few small dark cells are shown in the yolk.

FIG. 46. Stage *D*, later than in Fig. 45. The ectoderm thickening to form the stomadæum is shown at *o*.

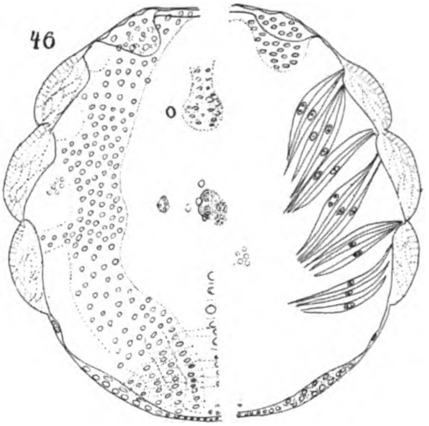
FIG. 47. The nauplius a short time before hatching.

FIG. 48. The nauplius just before hatching. In the left half of the figure the ganglia and mesoblastic rudiments of the post nauplius appendages are shown (stippled) in process of formation.

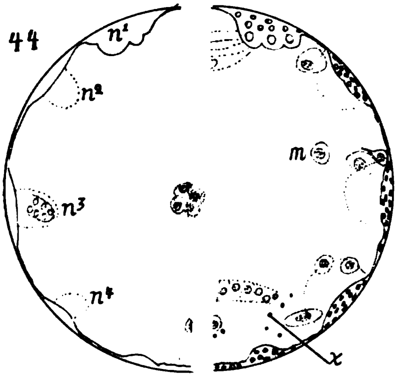
43



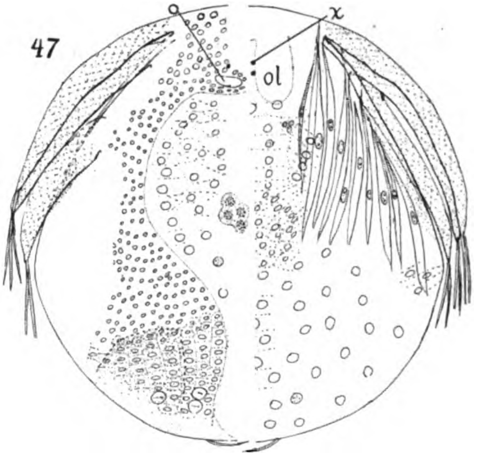
46



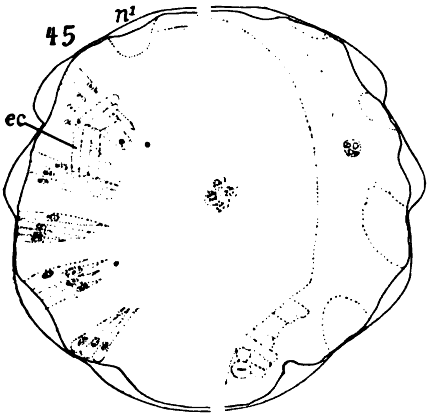
44



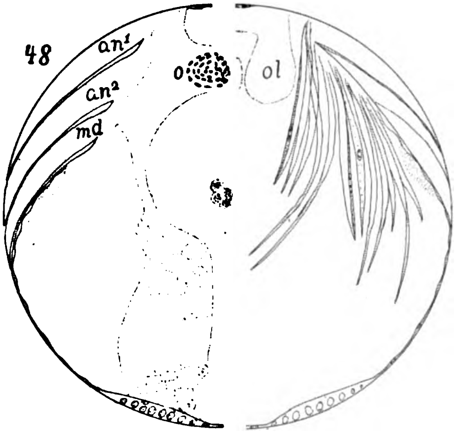
47



45



48



NEW SPECIES OF AMPHARETIDÆ AND TEREBELLIDÆ FROM THE NORTH PACIFIC.

BY J. PERCY MOORE.

Among the Polychæta dredged by the steamer *Albatross* while in the service of the Alaskan Salmon Commission of 1903 occur the following new species. Two previous papers based upon the same collections have been published in these *Proceedings* for 1905.

Amphicteis alaskensis sp. nov. (Plate XLIV, figs. 1–4.)

The available examples of this species vary in length from 22 to 76 mm. One selected for the type is 33 mm. long, of which the thorax is 19 mm.; its greatest width is 4.3 mm., and the cephalic cone is 2.3 mm. in both length and breadth.

Counting the region of trunk anterior to the paleoli as formed of two somites, the worm consists of thirty-four segments and the pygidium. The sêtigerous segments are from III to XX inclusive, and the uncinigerous VII to XXXV inclusive.

The median prostomial plate (fig. 1) is shield-shaped, about twice as long as broad, the posterior end pointed, the anterior cleft in the middle and formed of two somewhat divergent lobes. On each side of the plate is a broad, low, lateral ridge broadly rounded anteriorly and reaching not quite so far forward as the median plate. Bounding both these ridges and the plate posteriorly is a pair of transverse folds curving slightly forward laterally and caudad medially to meet in the median line at a sharp angle. These ridges are pigmented with brown above and they form the angle at which the nearly vertical anterior portion of the prostomium meets the nearly horizontal posterior region. The latter is much broader than the former and at its convex posterior margin nearly twice as wide as long. All of these parts taken together constitute the dorsal face of the prostomium. Beneath, and usually retracted within the mouth, is the folded tentacular membrane bearing a few short slender tentacles which scarcely exceed the cephalic cone in length. The somewhat quadrate prostomium is bounded by the short peristomial ring which completes the base of the cephalic cone.

The large mouth is bounded below by the prominent thickened peristomial lip, while dorsally the peristomium is much shorter and overlapped by the branchial ridge medially and more extensively by the paleolar tubercles laterally. This region is biannulate. The third somite is somewhat enlarged and tumid ventrally, the dorso-lateral portions project as thickened prominences bearing the paleoli, while the dorsal half is merely a low welt, strongly concave along its anterior margin and partly covered by the branchial ridge.

Somites IV and V are much shorter, together barely equaling III, and coalesced dorsally to form the ridge upon which the branchiæ are supported. The branchiæ form a group of four on each side so arranged that three are attached in a transverse anterior row on the region corresponding to somite IV and only one more posteriorly on V. Between the branchial scars of the two sides is a smooth quadrate area nearly twice as long as broad which is slightly inserted posteriorly into a transversely elongated area which reaches across the entire distance between the posterior pair of scars. A single branchia remains. This is slightly flattened, especially toward the tip, is regularly tapered to a slender end and its length about equals the greatest diameter of the thorax.

The next four or five somites increase slightly in both length and diameter, and then decrease in diameter but remian of constant length throughout the thoracic region. Each is divided into a dorsal and a ventral half-ring by the prominent setigerous tubercles. The latter have thick glandular walls and the intersegmental furrows are deep and well defined. The dorsal region is thin-walled and smooth, and the first five intersegmental furrows only are distinct.

Abdominal segments taper gradually to the pygidium and are nowhere distinctly defined by furrows. Dorsally the walls are high-arched, thin and smooth, ventrally they are thickened by a pair of prominent longitudinal muscle ridges with a neural groove between. Laterally between the parapodia of successive segments is a series of rugous areas, while across the ventral surface between each pair of parapodia a narrow glandular line frequently extends.

The pygidium is provided with a pair of thick lips bounding the vertical, slit-like anus and each bearing on the side an inconspicuous cirrus much shorter than the diameter of the pygidium.

Of the setigerous tubercles the pair bearing the paleoli are prominent and much larger than any others and they encroach much upon the lateral portions of the peristomium. The paleoli are about twenty (18-21) in number in each group and are inserted with their long diameters radial around an arc of nearly two-thirds of a circle. From this base they spread forwards and laterad in a broad, scoop-like figure.

The tubercles of IV and V are crowded closely together beneath the lateral margins of the branchial scars, that of the latter being slightly medial and caudal of the other. Both, and especially the latter, are minute. Succeeding tubercles up to the sixth increase in size, after which they remain of constant size and of a slightly flattened cylindrical form. Distinct, more or less club-shaped cirri are borne on the ventral side of the distal portion of all the tubercles, but they are larger and more abruptly clavate on the first four. The first and second tufts contain only six or eight setæ, the others twelve to fifteen, always arranged in two rows.

The uncinigerous tori are distinct, somewhat ear-shaped lappets arising from the posterior border of the segments below and separated by a short space from the setigerous tubercles. The anterior ones are the longest and their ends project most freely. Their size decreases regularly to the last thoracic. The uncini form a single irregularly curved line containing 160 to 170, which are largest dorsally and decrease regularly to the ventral end.

Abdominal somites are provided with shorter tori (fig. 2) which project more prominently than those of the thoracic segments. They bear about 75 uncini in a single series. No trace of ventral cirri can be detected, but the achætous notopodia are provided with prominent, curved, paddle-shaped cirri.

The paleoli form rather close spreading tufts, with the largest ones near the anterior margin but not reaching to the tip of the prostomium. They (fig. 3) are much flattened and moderately stout at the base and taper rather abruptly to acute tips. They are bright yellow and polished. The setæ are pale yellow, strongly striated, slightly curved, with narrow double wings and very acute tips. On the first three somites they are rather more slender than on the others.

The uncini (figs. 4*a* to *c*) are more or less triangular, with the upper rounded portion much elevated, the posterior ligament process very prominent, and the anterior one small and covered by the lowermost hook. The base is relatively short. The relative breadth to length varies. Usually there are six large, acute, closely appressed teeth or fangs, the clefts between which do not incise the base very deeply (less than one-half of the entire width of the uncinus). Frequently the upper tooth is much reduced in size.

The type of this species was taken with one other specimen at station 4,274, in Kadiak Bay, at a depth of 41 fathoms on a bottom of green mud with some fine sand. Another and larger one comes from Boca de Quadra, Southeast Alaska, in 48 fathoms and soft green mud.

Amphicteis alaskensis is easily distinguished from the species recorded in my paper of Japanese Polychæta, under the name of *A. japonica* McIntosh, by the character of the paleoli, which in the latter species are only ten or twelve, and when perfect have slender curved tips reaching far beyond the end of the prostomium.

Amphicteis glabra sp. nov. (Plate XLIV, figs. 5 to 8.)

The two known examples of this species are of moderate size, the type having a length of 23 mm., of which 15 mm. belong to the thorax, which is 3 mm. wide.

The form is rather slender, slightly clavate, and gently tapered. There are 20 thoracic segments, 17 of which are setigerous, and 15 abdominal segments, or possibly more as the extreme posterior end is macerated.

The entire prostomial region is relatively much broader than in *A. alaskensis* and its median plate (fig. 5) much wider than long, irregularly pentagonal, with the long anterior border cleft into a pair of short divergent lobes, from between which a broad median groove extends about halfway across the dorsal surface. On each side of this plate is a small triangular area, which is again bounded laterally by a narrow fold ending freely in front. The pigmented transverse ridges occur as in *A. alaskensis*, but are longer correspondingly to the width of the prostomium, which they cross nearly transversely; beginning at the posterior end of the lateral fold mentioned above they arch forward and meet at a wide angle in the median line immediately behind the apex of the cephalic plate. The entire dorsal surface of the prostomium lies in nearly the same plane, and its anterior portion is not bent downward at an angle with the rest as in *A. alaskensis*. The tentacular membrane is rather low and has a nearly regular elliptical outline, and the few tentacles remaining are scarcely longer than the entire prostomium.

The upper lip is slightly cleft medially and the peristomium in the region of the lower lip is enlarged and more or less tumid. Ventrally somite II is about one-third as long as the prostomium, and dorsally it exhibits a transversely elongated median area slightly elevated above and separated from the surrounding parts by a slight groove. Somite III is nearly as long as I ventrally; dorso-laterally it is prominently enlarged to bear the paleoli, and dorsally becomes very much reduced in length to form a narrow border that passes along the anterior margin of the branchiferous area and joins the median interbranchial area of IV. Succeeding somites are well differentiated and of nearly equal length throughout the thoracic region. The dorsal portion includes more than a semicircumference and is very smooth and iridescent. The ventral is much flatter, has deep bounding furrows and a transverse glandular line near the anterior margin. The ventral region slightly exceeds the dorsal in width and embraces it laterally so as to form a shelf or flange supporting the setigerous tubercles. The thoracic region tapers regularly into the abdominal, which differs from it little except in the form of the parapodia and the more prominent lateral muscles and deeper neural groove. The pygidium is much injured on both specimens.

Branchia have existed in the usual four pairs, but a single one only remains in place. It is nearly terete but somewhat compressed and tapers regularly from the base to the slender subulate tip. Its length is about equal to the width of the thorax or when appressed it reaches somite IX. The scars (fig. 5) show the branchiæ to have been more crowded than in *A. alaskensis* and to arise in a distinctly quadrate group, two pairs on each side. Apparently the two anterior belong to somite IV and the two posterior to V, and the two medial are somewhat larger than the two lateral. A shield-shaped area about twice as long as wide is present between the median branchiæ, reaching from the anterior margin of IV to the middle of V where it meets a transversely extended area limited laterally by the outer pair of branchiæ.

The paleoli arise from the dorso-lateral tubercles of III and form an open, spreading tuft, little concave and arising from a small arc. They number but eight or ten and all are slender and curved, with awn-like tips (fig. 7), and the longest reach beyond the tip of the snout.

The setæ tufts on the branchiferous segments are borne on small papillæ placed close together just laterad of the branchiæ. The setæ are few in number and much smaller than those on succeeding segments. Remaining setigerous tubercles are cylindrical and quite prominent with small truncate cirri, and bear compact tufts of about twelve setæ which are longer, more slender, and more curved than in *A. alaskensis*.

Uncinigerous tori are short inconspicuous lines near the posterior margins of the segments just below the setigerous tubercles. Posteriorly they become more prominent and on the abdominal segments project freely, but are usually so macerated in these specimens that the exact form is doubtful. The one represented in figure 6 shows the thickened dorsal cirrus.

The uncini (fig. 8) are narrow with very long base lines and five very slender acute teeth very deeply divided at the base. The anterior ligament process is rather conspicuous and lies beneath the lowermost tooth while the posterior ligament process is only moderately developed. The number of teeth is very constant and the dorsalmost are not so greatly larger than the ventral as in *A. alaskensis*. On somite X there are 115. Abdominal uncini are precisely similar, but owing to maceration the number on a torus is uncertain.

This species was taken at station 4,227 only, in Behm Canal, the depth being 62 fathoms and the bottom of dark green mud with fine sand.

A small portion of tube present is rather elastic and springy and is coated externally with a layer of brownish flocculent sediment.

Melinna cristata sp. nov. (Plate XLIV, figs. 9 and 10.)

The single example representing this species closely resembles *M. cristata* (Sars) Malmg., from which it differs especially in the more finely denticulated post-branchial membrane as well as in the much larger size and greater number of segments.

Although the posterior extremity is missing and the last one-third of the body is strongly spirally coiled, the specimen measures approximately 73 mm. long, exclusive of the branchiæ. At its widest part the thorax measures 3 mm., while the abdomen has a maximum width of 2 mm. The 17 setigerous thoracic segments have a length of 12 mm. and the fifty-five remaining abdominal segments 61 mm.

The prostomium or cephalic plate is a broad, short plate with a slightly convex crenulated anterior margin projecting freely over the bases of the fourteen to sixteen tentacles. The latter have a uniform diameter from base to end. The anterior and median ones are the largest with a length of about one and one-half times the thoracic width and about twice that of the posterior tentacles, which are scarcely as long as the body width and about half the diameter of the anterior ones. On the dorsal surface of the prostomium are the sensory folds, which are directly transverse. Beginning close to the lateral margins of the head they meet at the middle line and bend slightly forward side by side. Their dorsal surface is pigmented with a rich orange brown. Just in front of them on each side is a small, slightly elevated pad. The much folded upper lip is large and projects far beyond the mouth, just anterior to which is a distinct glandular area.

The lower lip is formed by the prominently projecting smooth margin of the prostomium, which is indistinguishably united to the first setigerous segment. Dorso-laterally it forms a prominent wing anterior to the first tuft of setæ, whence it is continued caudally into a flange-like ridge on each side of the thorax. Somites III and IV are well defined and together equal in length the single ring formed by the union of the prostomium and first setigerous somite. The remaining thoracic segments are equal and well distinguished on the ventral side, but thin-walled and coalesced dorsally. The ventral body walls between the tori are well provided with glands forming indistinct ventral plates on each segment. On the first four thoracic segments the flange-like ridges above the setæ are very distinct and, together with the pectinated membrane on somite V, bound a quadrate area in which the branchiæ arise. Anterior to the gills this region is marked by several slight transverse furrows. The postbranchial membrane is thin and deep and closely appressed to the dorsum of the thorax. It springs from the anterior border of V, and reaches nearly to the anterior border of IV, the free border being provided with eighteen blunt but prominent teeth.

The four pairs of branchiæ are permanently bent forward over the prostomium, and each has a length slightly exceeding twice the width of the thorax. They are all rather thick and coarse and taper from above the base to the bluntly pointed tip. For the basal one-fourth or one-fifth they are constricted and those on each side coalesced and so arranged that three lie externally, apparently arising from II, III and IV, and one internally on somite III. Between the latter, and uniting them across the middle line, is a thin membrane.

Somites II, III and IV bear short oblique lines of small setæ on the highest part of the lateral ridge, on the medial side of which and just posterior and lateral to the branchiæ, there is on each side of III a single very large claw-like spine. On V and all succeeding thoracic somites the setæ are in small tufts borne on truncate papillæ having the usual position. Each tuft contains about eleven or twelve setæ in two rows. Thoracic tori are constricted at the base, broad, truncate and beveled at the end. The uncini are borne on the thickest portion of the bevel, so that the free, thin margin projects a considerable distance beyond them. On somite X there are 63 uncini. Abdominal tori are narrow but much longer and of uniform width throughout, and the uncini are borne on the extreme unbeveled end. Somite XXV bears 36 uncini. The notopodial cirri are small and not conspicuously enlarged distally.

The setæ are all arranged in two equal rows. Those in one are larger, stouter and more broadly winged. Otherwise they are similar in both rows, and all have the stems slightly curved and strongly striated with the ends very acute and tapering. The isolated postbranchial spine is large and stout and has exactly the form figured by McIntosh for *M. cristata*.

The uncini (figs. 9 and 10) also resemble those of that species. Usually they have three teeth above the ligament process, but occasionally there is a fourth small or even well-developed one at the apex. Abdominal uncini differ from the thoracic only in their small size.

The tube measures 5 mm. in diameter with a lumen of half that size. The lining is a tough mucous membrane and the exterior a thick brittle coat of dark gray mud.

The type and only specimen was taken at station 4,258, in Lynn Canal, on a muddy bottom at a depth of 300 fathoms.

Artacama coniferi sp. nov. (Plate XLIV, figs. 11 to 13.)

This species is established upon two specimens measuring 22 and 45 mm. long respectively and neither of which is complete. The small one consists of 20 thoracic and 17 abdominal segments, is 3.5 mm. wide in the anterior part of the thorax and 2.2 mm. wide in the abdomen and posterior half of the thorax, and has the proboscis protruded for 3 mm. The larger example has 20 thoracic and 35 abdominal segments, with the caudal end still lacking. The proboscis is 6 mm. long, the thorax 17 mm. long, and its anterior half 6 mm. wide, while its posterior half tapers into the abdomen, which has a diameter of 2.5 mm.

The form is club-shaped with the first ten or eleven thoracic segments much enlarged, the middle of the thorax more or less abruptly contracted and the posterior portion tapered to the abdomen which continues to diminish to the posterior end.

The proboscis, which protrudes ventral to the mouth, is a large sugar-loaf-shaped organ as long as the first nine or ten segments, and has a basal diameter nearly equaling these. At the apex is a slender conical process about three times as long as thick, with its end rounded. Into it the retractor muscle is inserted, and it stands out prominently when the proboscis is extended. At the base the proboscis is somewhat rugous like the peristomium, with which it is continuous. Except for the rugosities the basal one-third is smooth, but the remainder bears small, low, rounded papillæ arranged in irregular rows, at first distant, but converging distally to the apical process, where they are more crowded, being separated by at most their own diameter.

The prostomium is folded into a double horseshoe-shaped oral fold with a broad, thin, median plate or membrane above the mouth, and behind this a pair of thick reflexed pads which become wider dorsally and nearly meet in the median line, and which bear the very numerous and much crowded tentacles. From the ventral end of the group, where they are mere papillæ, the latter increase until they almost equal the branchial filaments. Usually they are very slender with the ends enlarged.

The peristomium is longer dorsally than ventrally and is marked with longitudinal creases which are continued on to the base of the proboscis. Somite II is as long as the prostomium and equals the combined length of III and IV. Succeeding segments are again slightly longer and are distinctly annulated. In the posterior thoracic region the segments are divided by the setigerous tubercles into dorsal and ventral halves, the latter being again divided into a posterior larger and two anterior smaller annuli. Abdominal segments are annulated both dorsally and ventrally.

Three pairs of branchiæ occur on somites II, III and IV. Each is composed of numerous slender filaments having a length about one-third of the diameter of the body and sessile in a close tuft. The first contains about thirty, the second twenty-five and the third fifteen, but the number in each is probably greater in perfect specimens.

Ten ventral plates occur on the somites IV to XIII; the anterior ones are obscure, the posterior distinct. All are very short, about one-fourth to one-sixth as long as wide.

Setigerous tubercles number seventeen, and occur on somites IV to XX inclusive; though connected with the side of the body by an integumental fold they are very mobile, and the flattened, oblique tufts of setæ project prominently in various directions from near the dorsal end of the uncinigerous tori. Posterior tufts are broader and more mobile.

Anterior uncinigerous tori are long and narrow, extending over about one-sixth of the circumference of the body and separated ventrally by twice their length. Without becoming shorter they gradually approach ventrally until the intervening space is less than one-third their length. On the abdominal segments the tori are much more prominent and have a free ventral angle and a thin membranous wing arising from the dorsal border. This wing is gradually reduced posteriorly coincidently with a decrease in the size of the parapodium, but remains fairly prominent to the last.

What appears to be a slit-like sense organ is present on somite III below the gill. Small papillæ occur ventral to the setæ tufts of VI, VII, VIII and IX.

The setæ are all slender, nearly colorless, longitudinally striated, slightly curved and winged, and taper to very acute tips. In each tuft they are arranged in two rows of twelve to fifteen each, those in one row having longer and narrower wings (fig. 12), the others having them shorter and broader (fig. 11).

The uncini are arranged in a single series on the tori of somites V to X, are partly doubled on XI and completely in two series on XII to

XX. They are small and their number rather large, about 140–150 on V, 116 to 120 on X, about 110 in each series on XX and about 50 to 60 on the middle abdominal tori. They have the general form described for other species of the genus (fig. 13). The base is small and oblique, strongly convex below and with a prominent anterior angle, above which is a deep narrow sinus from which a slender process bearing the delicate, scarcely visible guard arises. There is no distinct neck, but a large head with a slender, acute, strongly decurved and slightly reflected beak, above which is the high, full crest with four cross rows of teeth.

No color remains.

The type comes from station 4,194, in the Gulf of Georgia, the depth being 111 fathoms and the bottom of soft green mud. The station from which the second specimen was taken is unknown.

Læna nuda sp. nov. (Plate XLIV, figs. 14, 15.)

This is another imperfectly known species, the only specimen available being a fragment of the anterior end measuring 21 mm. long and 1.5 mm. across the thorax, and comprising 28 anterior segments.

Projecting prominently from the anterior end is a broad, unfolded, scoop-like prostomial membrane curving gently and regularly upward from beside and above the mouth. Apparently the tentacles formed a complete series around the margin of the posterior limb of the prostomial membrane, but in this specimen most of them are gone and the three or four remaining are short and thick, and scarcely reach beyond the free border of the anterior prostomial membrane. Behind the tentacles the posterior prostomial fold is low and thick and at the dorsum nearly obsolete. On its posterior face are traces of a few eyes.

The mouth is a large, wide, transverse slit slightly bent down at the margins, and is bounded below by a low thick lip, and a prominent, broad, truncate process with slightly fimbriated margin springing from the middle of the peristomium.

Anterior somites are irregularly developed, the second being much larger than the adjacent ones. The second to eleventh somites inclusive have ventral plates, the glandular areas extending upward to include the setæ tufts. The third to the seventh have dorsal glandular areas also, which are separated on each side from the setigerous tubercles by a narrow non-glandular space. On the eighth also is a trace of a glandular region with a median break.

Not the slightest trace of gills exists. At the dorsal margin of III, in line with the setæ bundles of the following somites, is a prominent, stiff, erect cirrus on each side.

Behind the glandular region the segments become much longer, equaling or exceeding their diameter. These walls are very smooth and thin, highly arched above and nearly flat below, with thick longitudinal muscle bands along the ventro-lateral angles.

There are eleven setigerous somites, IV to XIV inclusive, but on the left side the bundle of IV is missing. Owing to the flatness of the dorsum of the region back to X the setæ tufts rise up nearly erect from the dorso-lateral margins. Though few, the setæ are long and, except on XII to XIV, conspicuous. Uncinigerous tori begin on V and are short and inconspicuous anteriorly, but about XV begin to increase in prominence, and on the posterior segments, while remaining short, project freely from the ventro-lateral longitudinal muscle bands. On the tori of somites V to X the uncini are arranged in one series, on XI to XIV in two apposed series. Each series on the thoracic somites contains about 55 uncini, while on posterior somites (XXX) only 40 occur.

The setæ (fig. 14) are slightly curved, delicate, with narrow wings, almost obsolete on one side and on the other frayed out and then abruptly ceasing, leaving a very acute almost whiplash-like tip. The uncini (fig.15) have the base broadly rounded in front and provided with a prominent triangular process behind. The sinus is narrow, and from its bottom rises a slender process bearing a very indistinct guard. The beak is rather slender and strongly curved, and above it is a very high crest composed usually of five or sometimes of six transverse rows, of about six or seven each, of long curved spines. The abdominal uncini have six or seven transverse rows.

The single specimen is a female filled with large eggs, and is preserved with a fragment of a soft mucous tube with a slight coating of foreign material.

It was taken at station 4,279, at Kadiak Island, at a depth of 29 fathoms and on a bottom of dark gray mud.

Thelepus hamatus sp. nov. (Plate XLIV, figs. 16 to 18.)

An apparently small species known only from the type, an incomplete specimen consisting of 31 anterior segments, and measuring 21 mm. long and 2.5 mm. in diameter at III, thence gradually diminishing caudally.

The tentacles number very few, less than 15 on each side, but are so fragile that they could not be safely untangled and the exact number ascertained. In the specimen the longest are 13 mm. long, coarse, thick and deeply folded longitudinally. The prostomial membrane forms a prominent horseshoe-shaped fold or upper lip above the mouth, while its tentacular fold is narrower and bears the tentacles in a nearly continuous band around its entire margin. On its posterior face are right and left groups of numerous, rather large, conspicuous, nearly black eyes arranged for the most part in one row and separated by a narrow dorsal space.

The large mouth is coverd by the upper lip, the inner surface of which bears a prominent grooved longitudinal ridge on each side of the middle line, and is bounded below by a thick slightly bilobed pad within the membranous anterior margin of the peristomium.

As far as about XX the anterior somites are short, the length not exceeding one-fourth or one-fifth of the width; those following are about two-thirds as long as wide, none is distinctly annulated, but all are rough and furrowed both above and below. The first four segments are glandular all round; the others have the thick glandular layer confined to the ventral half, but covering their entire length and extending to and including the setæ tufts. At about XVIII the glands disappear from the anterior one-third of the ventral surface also. The dorsum is thin-walled but rugous and constitutes less than one-third of the circumference.

Two pairs of branchiæ are developed and are situated dorso-laterally on II and III. The first consists of six and the second of three detached and independent filaments about as long as the diameter of the body; the area of attachment of the former extending over the entire length of the second segment, while that of the latter is limited to the anterior margin of the same dorso-lateral level of the third segment.

Setæ tufts begin on III and continue on all succeeding segments throughout the length of the specimen. All are small but prominent, little flattened and oblique, and are retractile within pits in the glandular layer of the skin. Throughout they are situated at a high level. Uncinigerous tori begin at V. They are all short, never exceeding one-sixth of the ventral interspace, and are separated from the corresponding setæ tufts by nearly their length. Posteriorly they grow smaller but more prominent. Like the setigerous tubercles they are situated in thin-walled, sunken spots within the glandular area.

The first seta tuft includes about twelve rather short, acute, slightly curved setæ with very narrow wings. In others the setæ (fig. 16) are more numerous, longer and more strongly curved. Still farther back in the posterior region they again become fewer. The uncini are arranged in a single series on all somites and number from 40 to 60.

They bear a close resemblance to Marenzeller's figures of the uncini of *T. cincinnatus* (Fabricius), but the height is greater in proportion to the length and the body and beak are more nearly equal in length. Those on the anterior segments usually have two teeth on the vertex of the crest above the beak (fig. 17*b*), but frequently there are three (fig. 17*c*) or even a fourth small one. The exact arrangement of the teeth in the crest varies, two of the varieties being shown in fig. 17*a*. Posteriorly the uncini (fig. 18) are smaller and the number of teeth in a transverse row of the crest usually more numerous.

No color remains in the specimen and no ova or sperm to indicate the sex. The fragment of tube present is membranous with scattered fragments of stone and siliceous sponge spicules.

The single specimen comes from station 4,235, at Yes Bay, in Behm Canal, at a depth of 181 fathoms and on a bottom of green mud.

Amphitrite palmata sp. nov. (Plate XLIV, figs. 19 to 22.)

Several specimens of this species occur in the collection. They resemble *A. affinus* very closely in most respects, but differ decidedly from that species in the form of the branchiæ, which have no elevated and branched base and no indication of dichotomy among the branches.

Complete specimens measure from 40 mm. to 88 mm. long, the last being 5 mm. wide across the thorax. The segments number from 62 to 64, those from V to XX being setigerous.

The general form is almost exactly as figured for *A. affinus* by Malmgren. Somites II, III and IV present similar slight lateral wings in successively ascending positions. The tentacles are moderately numerous and have a length of about five times the diameter of the thorax. No eyes can be detected on any of the specimens. The lower lip is square and rather prominent. Swollen glandular areas occur intersegmentally at the level of the gills on somites V to XIII; and ventral plates extend from III to XIII inclusive, the first three being very short. All of the segments are rugous and biannulate and taper gradually to the pygidium. The anus is a vertical slit surrounded by papillæ.

Most characteristic are the gills (fig. 22) which occur in three pairs on somites II, III and IV. Their bases are exceedingly short, so that the filaments are nearly sessile. They are expanded and flattened and the filaments spread regularly in a palmate fashion. The filaments are slender and when contracted are thrown into tight spiral coils. Full-grown specimens have 13 to 18 on the first, 10 to 12 on the second and 7 to 9 on the third gill.

Small papillæ or cirri are found just beneath the setigerous tubercles of somites VI, VII and VIII.

Setæ (fig. 19) have very short wings and a prominent fringed terminal pennant with a distinct knee at the place of its origin from the body of the seta. Abdominal uncini (fig. 21) resemble those of *A. affinus* quite closely, but those of thoracic tori (fig. 20) differ decidedly in the shape and proportions of the base and the number and arrangement of the teeth on the crest.

Some of the specimens are colored a nearly uniform reddish-brown, absent, however, from the gills and tentacles; and two are accompanied by portions of rather fragile tubes of fine mud.

This species was taken at the following stations: 4,227, Naha Bay, Behm Canal, 62–65 fathoms and on a bottom of dark green mud and fine sand; type locality 4,245, Kasaan Bay, Prince of Wales Island, 95–98 fathoms, dark green mud with fragments of shell, rock and sand; 4,253, Stephens Passage, 131 fathoms, rock and broken shells.

Explanation of Plate XLVI. XLIV

Amphicteis alaskensis—figs. 1 to 4.

Fig. 1.—Anterior end of type seen from the dorsum. × 7.
Fig. 2.—Parapodium of XXV. × 32.
Fig. 3.—Middle-sized paleolus; *a*, tip of another. × 32.
Fig. 4.—Three uncini from X; *a*, dorsal, *b*, middle (somewhat foreshortened), and *c*, ventral. × 585.

Amphicteis glabra—figs. 5 to 8.

Fig. 5.—Dorsal view of anterior end of cotype. × 12.
Fig. 6.—Parapodium of XXV. × 32.
Fig. 7.—Middle-sized paleolus. × 45.
Fig. 8.—Uncinus from X. × 585.

Melinna denticulata—figs. 9 and 10.

Fig. 9.—Uncinus with 3 teeth above the ligament process from X. × 585.
Fig. 10.—Uncinus with 4 teeth from the same torus; the anterior end of the base is broken. × 585.

Artacama conifera—figs. 11 to 13.

Fig. 11.—A broad-bladed seta from X. × 585.
Fig. 12.—A narrow-bladed seta from X. × 585.
Fig. 13.—An uncinus from X. × 800.

Læna nuda—figs. 14 and 15.

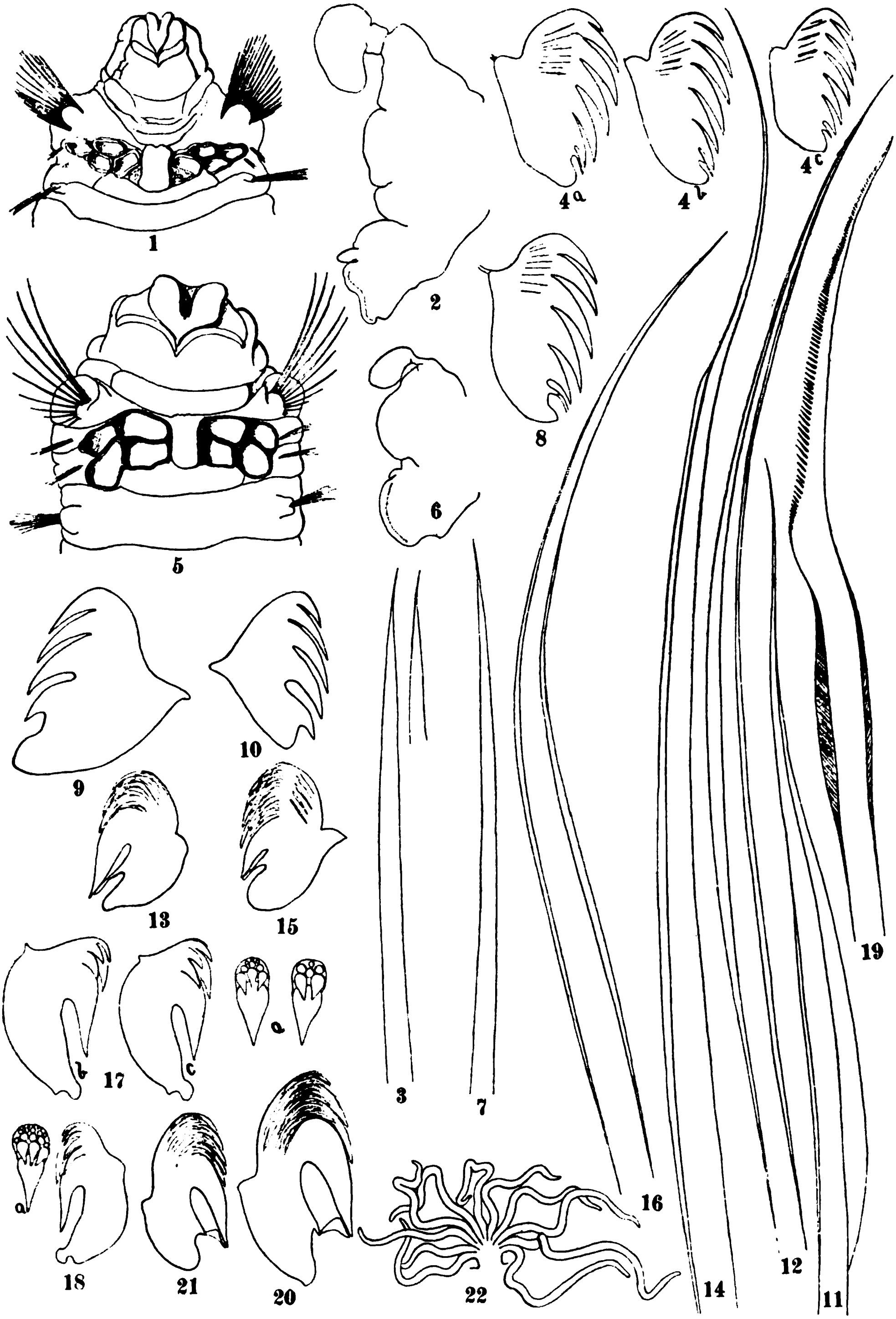
Fig. 14.—Seta from X. × 585.
Fig. 15.—Uncinus from X. × 800.

Thelepus hamatus—figs. 16 to 18.

Fig. 16.—Seta from X. × 585.
Fig. 17.—Two forms of uncini from X; *a*, front views of two similar ones showing different arrangements of the teeth. × 585.
Fig. 18.—Uncinus from XXX; *a*, front view of another. × 585.

Amphitrite palmata—figs. 19 to 22.

Fig. 19.—Seta from VII. × 335.
Fig. 20.—Uncinus from XX. × 480.
Fig. 21.—Uncinus from L. × 480.
Fig. 22.—One of the second pair of gills. × 8.



MOORE. AMPHARETIDÆ AND TEREBELLIDÆ.

ADDITIONAL NEW SPECIES OF POLYCHÆTA FROM THE NORTH PACIFIC.

BY J. PERCY MOORE.

In the following pages are described a number of new species of Polychæta, belonging to several families, from the collections of the Alaskan Salmon Commission of 1903. Three papers noticing other novelties in these collections have already been published in these *Proceedings* with the approval of the Commissioner of Fisheries, through whose kindness the material was placed in my hands for study. The full report on all of the species represented will appear in the Bureau of Fisheries *Bulletin.*

Notophyllum imbricatum sp. nov. Plate X, figs. 1–3.

The two examples upon which this species is founded bear a remarkable resemblance in proportions and general aspect to a *Polynoe.* The body is depressed and the large, scale-like notopodial cirri are imbricated and, except for a short space near the anterior end, completely conceal the back from above.

The type and larger specimen is a trifle over 30 mm. long, and the greatest width between tips of the parapodia a little anterior to the middle of the body is 6.5 mm. The small individual is 12 mm. long and 1.8 mm. wide, and the back is concealed by the elytra even more completely than in the larger one.

The prostomium is flattened and wedge-shaped, with the slightly concave or straight base about two-thirds of the length, and the angles rounded. A slight emargination of the anterior portion of the sides is filled by an irregular low tubercle from which the frontal cirri arise. A pair of large brown eyes occupy the greater part of the posterior region of the prostomium laterad of the median tentacle (fig. 1).

The frontal tentacles arise in lateral and ventral pairs, the latter slightly in advance, from the slight emargination above mentioned. They nearly equal the prostomium in length, are stout and somewhat swollen above the base, and taper to fine tips. The median tentacle arises between the eyes and nearly fills this space. It is one and one-half times as long as the prostomium, stout and somewhat enlarged above the base and tapers to a slender end. From behind its base a low ridge runs to the posterior margin of the prostomium. In both specimens the proboscis is retracted and the large mouth is bounded by a rather prominent lip, formed by the union of the peristomium with the succeeding somite.

The peristomium and two succeeding segments are much crowded forward so that the tentacular cirri arise beneath the head. The first or peristomial cirrus is a ventral cirrus and arises from the forwardly directed portion of the peristomium beneath the eye. It resembles the median cephalic tentacle in form and size, but is a trifle longer and arises from a distinct basal article. Arising from beneath the posterior dorsal margin of the prostomium, apparently from the peristomium, are three somewhat flattened appendages on each side, the outermost of which is the longest and connected with the corresponding tentacular cirrus by a slight web. The middle one is nearly as long, and the inner one is minute (fig. 1).

Somite II bears two pairs of tentacular cirri, a dorsal and a ventral one, separated by a considerable interval in which arises a small papilla probably representing a parapodium. The ventral cirrus occupies a position below the peristomial cirrus, which it resembles, but which it exceeds decidedly in both length and thickness. The dorsal cirrus of this somite arises at a higher level than any others in the body from beneath the postero-lateral angle of the prostomium, and its rather stout but long and tapering style reaches to IX or X. Somite III bears a dorsal tentacular cirrus only, which, with the complete parapodium to which it belongs, is depressed to a position more ventral than usual, in marked contrast to the dorsal cirrus of II. It arises from beneath and slightly behind the latter, which it resembles in form and size (fig. 1).

As stated before, the first three somites are carried well forward, the peristomium and II being coalesced ventrally but distinct, though very short, above. Remaining somites are well differentiated and obscurely biannular, the anterior and decidedly larger annulus bearing the parapodia. There are about 78 somites, the posterior end of the body being in a state of regeneration and the somites of that region as a consequence very small and tapered to a minute pygidium.

The body, excluding the parapodia, is slender, about two-fifths of the total width, and of nearly uniform diameter, except near the tapering ends. It is somewhat arched dorsally; flattened and with a shallow neural groove below.

Parapodia (fig. 2) are prominent and well developed throughout.

In the middle region they exceed one-fourth of the entire width. They consist of a broad base, a somewhat flattened, tongue-shaped neuropodium, slightly cleft at the tip and bearing a rounded and swollen prominence on the ventral side of its base, and a minute papilliform notopodium, which diverges dorsally from the neuropodium. Each division is supported by a single aciculus, the notopodial being very slender and curved, the neuropodial stouter and straight.

Along the posterior face of the base of the neuropodium above the papilla mentioned arises a flange-like ceratophóre bearing a large, foliaceous palette-shaped ventral cirrus which curves upward behind the neuropodium and completely conceals both it and its setæ from behind. The notopodial ceratophore is very large, causing the notopodium itself to appear as a mere appendage. At its dorsal side the free distal border is prominently produced, thus prolonging the surface of attachment of the notopodial cirrus. The cirrus itself is very prominent, of a somewhat irregular reniform outline and attached by the marginal sinus. All of the notopodial cirri are turned nearly horizontally and overlap on the back in an imbricate fashion, closely similar to the elytra of the *Polynoidæ*. Anteriorly the dorsal cirri diminish in size so that they fail to cover the back completely and their ceratophores become more slender and elevated. The ventral cirri also become smaller, but remain prominent as far forward as III without essential change of form or position. A minute neuropodial tubercle exists on II, but it is uncertain if setæ are present thereon.

The eyes are purplish brown and the general color of the body a distinct dull greenish olive. Little pigment remains in any part of the body, but the notopodial cirri are more or less marked with dusky streaks and spots. A few small ova float free in the body cavity.

The notopodium usually bears but a single slender, curved and simple seta, and even this appears to be absent from several of the anterior parapodia. Neuropodial setæ are numerous, upwards of 20 to 30 occurring in the subacicular and 12 to 18 in the supraacicular groups. They are colorless and transparent, compound, with the stem gently curved and slightly enlarged at the end, where each side of the socket is provided with 6 to 8 very long, slender teeth and several shorter ones. The blade is slightly curved and tapers to an acute tip, and is striated and provided with minute marginal denticulations (fig. 3).

The only specimens are the two from Station 4,269, Afoqnak Bay, 14 to 19 fathoms, hard gray sand and rocks.

Eulalia quadrioculata sp. nov. Plate X, figs. 4–6.

This description is based upon a single much contracted entire specimen (type) and a fragment of the anterior end of another in every way similar.

The type is 27 mm. long, the protruded proboscis 2 mm. additional, the width without parapodia 2 mm., with parapodia 3 mm., the diameter of the distal end of the proboscis 2.5 mm., and the number of segments 106.

The evidently much contracted prostomium (fig. 4) is about two-thirds as long as wide, scarcely emarginated in the median line posteriorly, rather tumid in the posterior lateral part and then slightly concave to a small truncate median anterior lobe. The usual pair of eyes are transversely elliptical, situated on the dorsal surface of the prostomium about three times their diameter apart, nearly twice their diameter from the lateral margins of the head and not more than their diameter from the posterior margin. They have distinct lenses. In nearly the same transverse line or very slightly in advance and half way between the dorsal eyes and the margin, or just within the nuchal organs, is a second pair of eye-like spots of black pigment, but lenseless and smaller and more irregular than the dorsal eyes.

The four frontal tentacles are subequal, about as long as the head, and rather thick, with acute tips. The ventral pair project somewhat downwards, the dorsal directly outwards. From the middle of the small lobe lying between the frontal tentacles a shallow longitudinal groove passes to the median tentacle, which arises from a point just anterior to the eyes. It equals the frontal tentacles in length, but is slightly more slender in its distal part. The four tentacular cirri arise in the positions usual in the genus from somites I, II and III, and are short and subequal, being about twice the length of the prostomium.

The segments are all well differentiated and dorsally are strongly arched; anteriorly they are strictly simple smooth rings, but in the posterior third become biannulate. Ventrally the body is marked by a neural groove and lateral ridges bearing glandular areas ventral to the parapodia. On this surface the biannulation extends nearly to the anterior end. The anterior segments are contracted and extremely short, farther back they become relatively longer, and toward the posterior end the body is distinctly flattened. The pygidium, which is provided with a thickened welt-like rim surrounding the anus, bears on the ventral side a pair of prominent acuminate cirri resembling the ventral cirri in size.

Parapodia are located at the level of the ventral surface. That on

II, related to the second tentacular cirrus, is rudimentary and achætous; that on III is larger, perfectly formed and bears setæ. The others have the form shown in fig. 5 and bear foliaceous dorsal cirri. When fully developed they are sharply marked off from the ventral glandular swellings by distinct lateral grooves. The notopodium is of course wanting, and the neuropodium is a small somewhat flattened process divided distally into a very small postsetal and a decidedly longer presetal lobe, from between which the single vertical series of setæ projects. Each lobe is notched on the edge at the point where the single straight slender aciculum reaches the surface. The neuropodial cirrus is prominent from III back and is a short, rather thick process attached to the posterior side of the base of the neuropodium. In the middle region it is somewhat foliaceous and reaches to the tip of the neuropodium; in form it is triangular with the broad, somewhat convex base ventral and the apex dorsal, while the attachment is by one of the short sides. Posteriorly they become more slender and project distinctly beyond the end of the neuropodium.

The dorsal cirri (fig. 5) are prominent and foliaceous throughout. They arise from stout bases situated a short distance dorsal to the neuropodia, which they in most cases exceed in size. All of the cirri have their fibrous and glandular structures arranged pinnately along an axial core. Anteriorly the cirri are rather broadly lanceolate with acuminate, somewhat recurved tips; posteriorly they become more slender and elongated; and finally are very narrowly lanceolate and of a length exceeding the diameter of the body. At the same time their foliaceous character is gradually lost.

About thirty setæ, equally divided between the supraacicular and subacicular groups, form the vertical fan-like fascicle. They are of the usual compound form (fig. 6) with the transparent, colorless stems rather stouter than those of *E. longicornuta*, the thickened and nearly truncate end furnished on each side with seven or eight slender teeth, one of which is much larger than the others. The blades are short, broad at the base, and rather conspicuously striated and fringed.

The type specimen, a female filled with large eggs, retains a dull olive color throughout the body, becoming brown on the dorsal cirri. Besides the black pigment in the eyes, there is a diffuse spot near the tip of each dorsal cirrus, and a very minute spot beneath each ganglion of the ventral chain. The cotype shows some indications of a faint transverse band across the dorsum of each segment.

The type and cotype are from Quarantine Rock, Port Townsend, Washington, June 27, 1903.

Eulalia longicornuta sp. nov. Plate X, figs. 7, 8.

A complete example, much contracted, measures 15 mm. in length, with a body width of 1.5 mm. at the widest part, and a total of 73 segments.

The broad prostomium is very slightly cordate, about as wide as long, and has no distinct concavity or constriction behind the frontal tentacles. The latter arise from the extreme anterior end of the prostomium, are slightly longer than the head and very slender. The median tentacle arises immediately in front of a line connecting the anterior border of the eyes or very close to the centre of the prostomium. It is 1⅔ times as long as the head, very slender, and tapers regularly from the base to the tip.

The single pair of eyes are circular, black, situated nearly their diameter from the posterior margin of the prostomium and twice their diameter from each other. Both specimens have the proboscis retracted, in which condition the mouth is bounded below by a somewhat swollen, longitudinally furrowed lip. The peristomial somite appears on the dorsum as a slightly elevated lenticular area overlapping the prostomium. The first ventral tentacular cirrus arises directly beneath the eye, the second one from somite II; both are slender and subulate and reach back to somite VII or to a length 2½ times the head. The two dorsal tentacular cirri arise from II and III respectively, and are about twice the length of the ventral cirrus, very slender and regularly tapered to a delicate tip.

Dorsally the body is strongly arched above, below flattened and slightly grooved. It is widest at the middle and tapers regularly and nearly equally both ways. The segments are well marked throughout and show scarcely a trace of biannulation, even posteriorly. The anal cirri are missing from both type and cotype.

Although the specimens are smaller, the parapodia (fig. 7) are even more prominent than in *E. quadrioculata*, owing to the projecting character of the dorsal angle of the presetal lobe, but otherwise they are similar. The dorsal cirri are, however, very different from those of that species, being folded against the sides of the body instead of held erect. They are strongly foliaceous throughout, the anterior and middle ones having a broadly pyriform outline, and those of the latter region being especially broad; the posterior ones are more slender and rather cuneate-ovate. Moreover, the central area is always broad and thick and the gland ducts and other markings radiate from it in all directions and not in a pinnate manner. The ventral cirrus is also prominent and projects beyond the dorsal lobe of the parapodium.

There are about 10 supraacicular and 12 subacicular setæ in all, but fewer on the extreme anterior and posterior parapodia and, except for the usual variations in relative length of the blade, all have the form exhibited in fig. 8. The slender stem ends in an acutely oblique enlargement with 7 to 9 nearly equal, closely appressed teeth on each side. The blades are slender, flexible and very acute, with very fine marginal fringe.

In both specimens the color has faded to a nearly uniform olive with some brown striations on the dorsal cirri. The type is a female filled with eggs. These specimens were found among serpulid tubes taken at the Quarantine Station dock near Port Townsend, Washington, on June 27, 1903.

Pionosyllis magnifica sp. nov. Plate X, figs. 9–11.

This large syllid is described from two specimens, one of which (the type) measures 48 mm. long and nearly 2 mm. wide, exclusive of cirri and setæ, in the middle of the body. The form is much depressed, especially in the widened middle region, from which it tapers to the very small head and pygidium.

As just indicated the prostomium is small, its width little exceeding three-fifths of the width of the second segment and one-fifth of the maximum breadth of the body. It is depressed, somewhat quadrangular in form, widest anteriorly where the width is about double the length. The somewhat flattened palpi project forward and somewhat downward and are very slightly connate at the base (fig. 9).

All three of the cephalic cirri are decidedly slender and arise in a transverse row from almost the extreme anterior margin of the prostomium. In one specimen they are subequal and about three times the length of the prostomium. In the other the median one is three and one-half times, the lateral about twice the prostomial length. The ends may be partly sloughed away.

The eyes, though small, are very conspicuous and nearly black, the anterior slightly the larger. Together they form a quadrate figure conforming to the outline of the head and about two-thirds as large.

Dorsally the peristomium is very short, but ventrally it projects as a prominent lip surrounding the large mouth. A fragment of the anterior end of an example of this species from Station 4,235 has the proboscis protruded as a short bell-shaped structure, bearing ten prominent papillæ at the end and just behind them a conical, dorsal median tooth which appears to be quite soft. On the roof of the pharynx just behind the everted portion there appears to be, however, a hardened, horny elliptical area. This specimen also has the eyes larger than the others and nearly connate, but no swimming setæ exist on any of the small number of anterior segments present.

Of the tentacular cirri (fig. 9) the dorsal peristomial is about four times as long as the prostomium, that of somite II is slightly longer and of III as much shorter; the ventral peristomial cirrus is about two and one-half times the prostomial length. All of the tentacular cirri resemble the cephalic cirri in being slender, delicate, and not moniliform, or with indistinct irregular furrows only.

After the second, the segments increase very gradually in length and more rapidly in width and soon assume the depressed form characterizing the middle region where they are five or six times as wide as long. The type specimen has about 150 somites, the cotype only 110. Posteriorly the body becomes slender and ends in a minute annular pygidium, bearing on its ventral side a pair of very slender caudal cirri equalling the last twelve segments and exceeding any of the cephalic appendages.

The parapodia are of the form usual in Syllidæ and project prominently from the sides at a low level. Although there is no distinct notopodium, a slender notopodial aciculus is always present just beneath the notopodial cirrophore (fig. 10). The well-developed neuropodium terminates in a broadly rounded, more dorsal, postsetal process and a longer, rather prominent, and more ventral presetal process. The three or four aciculi terminate at the upper outer angle of the former. Ventral cirri are always short, stout and bluntly rounded, with oblique bases passing into the ventral surface of the body, and in size about equal the neuropodia. Notopodial cirri arise from very large and prominent cirrophores. Except for their slightly larger size anteriorly, where they are about three times the length of the prostomium, the notopodial styles are similar throughout. They are probably somewhat contracted and in life would be longer and more slender. In the middle of the body they are scarcely one-half of the total width. As shown in the figure they are rather stout and coarse, and, though more or less deeply marked with irregular transverse furrows, are never regularly articulated or moniliform. The only variation in the parapodia is that they become more prominent in all their parts posteriorly.

Notopodial aciculi are slender, curved and acutely pointed; the neuropodial are stouter, nearly straight and knobbed at the end. All setæ (fig. 11) are compound and all are subacicular in position. In middle parapodia they are numerous, arranged in about ten horizontal rows of three to five each, or about forty in all. They are colorless, with long, slender, curved shafts, the ends of which are rather abruptly enlarged and not very oblique, and are provided with only a few small teeth at the apex. The appendages or blades are strongly hooked and bifid at the end and distinctly fringed. The shortest posterior ones have a length of about one and one-third times the diameter of the end of the shaft, the longest about four and one-half times that diameter.

Both specimens are entirely colorless and the large one is filled with masses of sperm.

The type comes from Admiralty Inlet, near Port Townsend, Washington, Station 4,219, 16 to 26 fathoms, on a bottom of green mud with sand and broken shells. A fragment was taken at Yes Bay, Behm Canal, 130 to 193 fathoms, bottom of gray mud.

Stauronereis annulatus sp. nov. Plate X, figs. 12, 13, and Plate XI, figs. 18–22.

The larger example (much contracted) is 13 mm. long and about 1 mm. wide exclusive of the setæ, and has 72 segments. The other is 9 mm. long with 62 segments.

The prostomium (fig. 12) consists of a broad shovel-like anterior process and two short segments, each bearing a pair of tentacles and a pair of eyes. Although these two divisions or rings are about equal, both the eyes and tentacles of the anterior one are much the larger. The anterior tentacles (palpi) arise from the ventro-lateral region of the first annulus. Each consists of a large and very stout basal piece strongly curved backwards by the sides of the head and bearing on its end a very small ellipsoidal terminal article. Just above the base of each, on the dorso-lateral region of the head, is a large very dark brown eye. Immediately behind and slightly above the large eyes the second pair of tentacles are borne on the second annulus. They are about as long but not so stout as the anterior ones, are cylindrical in form and consist of six or seven nearly spherical articles. The second pair of eyes are minute dark brown spots about one-fifth the diameter of the anterior pair, and are situated on the dorsum of the second annulus about half as far apart as the anterior pair. The mouth is small and bounded behind by the second somite, and from it project the ends of the jaws (fig. 18). Owing to the retracted state of the proboscis the jaw apparatus is not all visible. A specimen cleared in glycerine shows on the dorsal side a lenticular area having a very dark brown border and a paler interior enclosing a small central space. In ventral view are seen the pair of dark brown mandibles (fig. 18) with slender bowed bases and curved, divergent, tapering end-plates bearing about seven strong teeth along the medial margin.

The prostomium and somite II are apodous, short simple rings. All others bear parapodia, but are not otherwise more complicated in structure. Up to about the fifteenth they increase in size, but remain uniannular throughout. The body is strongly arched above and nearly flat below, and terminates in a simple ring-like pygidium bearing a pair of small ventral anal cirri as long as the diameter of the pygidium.

Parapodia are small, slender and strictly lateral in position. Except that they correspond in size with the segment bearing them and are consequently largest at the middle of the body, they are quite similar throughout. The neuropodium (fig. 13) is slender, nearly cylindrical and slightly enlarged distally, where it terminates in a presetal lobe divided into a larger ventral and a minute acicular process, and a postsetal lobe which begins just below the aciculum and runs to a rather prominent dorsal angle. Its dorsal surface bears a group of long cilia. The neuropodial cirrus is a simple finger-like papilla arising in or near the distal third of the ventro-posterior surface of the neuropodium and reaching nearly to the end of the latter.

The dorsal cirrus arises immediately above the base of the neuropodium and consists of a slender, elongated cirrophore nearly as long as the latter and slightly diverging from it. Usually but not always it is slightly constricted about the middle and the end is a little thickened. Probably it represents the notopodium, as what appears to be a slender aciculus penetrates about half of its length. A tuft of long cilia resembling that on the dorsal surface of the neuropodium is usually present on its ventral and sometimes on its dorsal surface also. On the end of this basal piece is borne a terminal style of about half its length and of a slender, conical form. The first parapodium altogether lacks a dorsal cirrus.

Setæ are numerous and of four forms, all very delicate, transparent and colorless. They are arranged in well differentiated supra- and subacicular fascicles, each comprising two kinds, and all of which occur throughout the body, except perhaps in a few anterior segments, where the long compound setæ have not been found.

All of the supraacicular setæ are simple; those most numerous being very delicate but stiff capillary bristles, tapered to very acute tips, slightly curved and rather strongly serrated in an antrorse manner along one side (fig. 20). Their exposed parts are fully three times as long as the entire neuropodium. The other form has generally stouter stems, straight and tapering until near the end (fig. 19), where they present a gentle ventral curvature and at the same time become broadened and end in two divergent, slightly curved points, the ventral of which is the longer; while below the dorsal is a serrated sheath, the rather coarse teeth of which are directed outward. Seldom more than three or four of the latter form occur in a parapodium along with ten or fifteen of the former.

Subacicular setæ are all compound. The most numerous kind occur to the number of about fifteen. They have moderately stout, rather strongly curved stems, the end being very unequally and obliquely bifurcated to form a socket, the dorsal border of which is provided with a few teeth (fig. 20), while the blades are comparatively short, but increase in length from the ventral to the dorsal margin of the bundle, and have one border fringed and the end terminated by a pair of distinct but not widely separated and nearly parallel teeth. The second kind of compound setæ (fig. 22) seldom exceeds three in number. They have the same construction as the more numerous form but are much more slender and delicate in all their parts, and the blade often equals the entire length of the neuropodium or about three times the length of the longest blades of the other type. On the most anterior segments the two forms appear to grade into each other. The setæ of this species differ decidedly from those of typical members of the genus and are more nearly like those of *Prionognathus ciliatus* Keferstein.

These worms are quite colorless and their form and histological structure indicates that they may be pelagic in habit.

The type and a somewhat smaller cotype were obtained at Quarantine Rock, Port Townsend, Washington, June 27, 1903.

Notomastus giganteus. Plate X, figs. 24, 25.

The only complete specimen measures 140 mm. in length and 7 mm. in maximum diameter in the thoracic region, but a second incomplete example is much larger. Even taking into account the contracted state of the specimens, this species is much stouter than usual for the genus. The body is nearly terete or slightly depressed and for the first fifteen or twenty millimeters increases in diameter, and then falls off to the posterior end which is two millimeters in diameter within ten segments of the anus.

The prostomium is a small rounded lobe bearing a minute conical palpode and is completely retracted within the peristomium, which, except in being slightly longer, resembles the immediately following segments. The protruded and collapsed proboscis forms a discoid, wrinkled structure fully 8 mm. in diameter. All of the thoracic segments are strongly biannulate, the anterior annulus being slightly shorter than the posterior. Each annulus is marked out in irregular areas, of which there is generally but one series to each annulus, though on somites IV to VII inclusive they become arranged irregularly in two rows. Behind VII the thoracic segments become smooth and more glandular and decrease in length, though even the last is fully one-half longer than the first abdominal.

Beginning with II, each thoracic segment bears small notopodial and neuropodial tufts of very delicate, narrowly winged capillary setæ in the usual positions, but neither the lateral sense organs nor the genital pores can be detected in surface views of these specimens.

The abdominal segments, of which there are 190, are very short, with shallow, ill-defined furrows, and are either simple rings or anteriorly obscurely biannulate. The surface is smooth, and the integument provided with a thick glandular coat on the anterior and a much thinner coat on the posterior segments.

The notopodial tori are very long, but not at all elevated above the surface in these specimens, and bear a great number of uncini. The notopodial torus is much more elevated and prominent, especially on posterior segments, but is much shorter and contains a much smaller number of uncini. The gills are low, rather long, inconspicuous folds. The pygidium forms a narrow circumanal welt bearing two longer prominently protruding ventral cirri and four much smaller ones in two pairs more dorsally placed.

The uncini (figs. 24, 25) are numerous and very delicate crochets of a peculiar form. They are f-shaped with the densely fibrillated core exhibiting a slight spiral turn, and the tip provided with a single bent terminal process, somewhat flattened and at the base swollen and overarched by a depressed hood, the margin of which is denticulated with eight or ten teeth.

The type comes from Station 4,264, off Freshwater Bay, in Chatham Strait, at a depth of 282–293 fathoms, and on a bottom of green mud; the larger but incomplete cotype was taken at Station 4,197, in the Gulf of Georgia, at a depth of 31 to 90 fathoms, on a bottom of sticky green mud and fine sand.

Travisia pupa sp. nov. Plate XI, fig. 23.

This is a thick, stout, grub-shaped worm tapering nearly equally both ways but having the anterior end rather blunter and thicker. The considerable number of examples in the collection measure from 24 mm. to 82 mm. in length, with corresponding diameters at the middle of from 8 mm. to 32 mm. Exclusive of the pygidium there are thirty-one or thirty-two somites, but the number is not correlated with the size of the worm, the largest two having thirty-one and the smallest thirty-two.

The prostomium is a minute conical organ, thin-skinned, weak-walled and hollow, and apparently capable of distention by internal fluid. It is followed by a short, uniannular segment continuing the general conical form of the anterior end. Dorsally this segment is crenulated on its posterior margin; below it is flattened and slightly grooved in the middle of the posterior part; and on each side it is provided with a dorso-lateral groove which terminates anteriorly in a deep sensory pit at the posterior margin of the prostomium. The next segment is biannular, with the anterior ring distinctly larger. Below it forms the upper lip and is thrown into a number of deep longitudinal folds and furrows which pass into the mouth. On each side, above a rather prominent swelling, is a quadrangular area opposite the groove on the first segment and itself bounded by a longitudinal groove above and another below, both of which cut the segment for its entire length. This area bears the small tufts of notopodial and neuropodial setæ and between them an elliptical clear spot or pit. Dorsally each ring is marked by longitudinal grooves which effect a peculiar lobed and crenulated arrangement of the posterior margin. The third somite is triannulate, but the anterior two rings are somewhat united and on the ventral surface the first enters the sides of the mouth and the second forms the longitudinally grooved lower lip. At the sides this segment is constructed like the second, except that the posterior third of the quadrate area is depressed and smooth and that a cirrus is borne on each side immediately above and behind the notopodial setæ. Above the same longitudinal sulcation and posterior lobing appear. The next twelve segments are formed of three equal rings completely separated by continuous furrows, except for the short interruptions at the setigerous areas on each side. Dorsal longitudinal sulcations are wanting and a second sensory pit, which first appears on the middle of the second ring below the setigerous area of somite IV, becomes very conspicuous on the posterior segments of this region, and on every specimen finally ceases on XV. Somites XVI, XVII and XVIII each consist of a large posterior and a small anterior ring, the furrow separating which becomes successively more and more restricted to the dorsal and ventral regions. Traces of a short anterior ring, differentiated only dorsally and ventrally, still continue on XIX and XX, but all remaining somites are strictly uniannulate with prominent overlapping posterior margins, which finally become telescopic. Somite

XIV, which is exactly at the middle of the body, is the longest; but the reduction in both length and diameter of the segments is slight until near the ends, where it becomes more rapid. The pygidium is a short squarely truncated tube marked externally by longitudinal grooves which correspond with the clefts between the nine to twelve uneven lobes into which its margin is divided.

The surface of the body is vesiculated or finely pustular in the following manner: Generally over the posterior half of the body, in all of the intersequental furrows and on all except the most anterior setigerous areas, the pustules are very small and, though numerous, not crowded. In macerated specimens they are collapsed and appear as punctations. From the middle of the body they gradually increase in size forward. Each annulus of anterior somites is provided on its highest part with an irregular transverse series of very large vesicles which usually lie nearer to the posterior margin and overlap the succeeding ring, when the worm is contracted, as a rough and irregular fold interrupted in the median dorsal region. These folds and their vesicles are usually best marked on the middle ring of each somite, on which they increase in prominence from the median break laterally to the setigerous areas, below and even more above which they form rough lobes overlapping the third ring. The ventral lobe bears the ventral sensory pit referred to above. Anteriorly the transverse series of enlarged vesicles tends to form several rows and all of the vesicles to increase in size. The first three segments bounding and anterior to the mouth are covered nearly uniformly with vesicles of moderate size; and similar ones cover the anterior two-thirds of the setigerous areas of the first ten or twelve segments. The prostomium is perfectly smooth and lacks surface vesicles altogether. Posteriorly the ridges of enlarged vesicles and the lateral lappets become gradually flattened out and the entire surface much smoother. The lappets above and below the setigerous areas remain, however, and may become even more prominent on the last few segments, where, however, they are not vesicular.

Neuropodial cirri appear on III and continue on every segment to near the posterior end, ceasing on from XXIV to XXVII in different specimens. They arise at the dorsal margin of the setigerous area, chiefly from the third annulus but in part from the second also. At the base their diameter nearly equals the length of the third annulus, but they quickly become slender. The surface is strongly wrinkled transversely, much like the contracted tentacle of a jelly-fish, and doubtless they are in life capable of great extension; but in the preserved specimens, even where longest (in the middle of the body), they barely equal one-half or one-third of the body diameter. Toward the ends they are reduced to one-third or even one-fourth of this length.

Lateral sense organs appear as a pair between the prostomium and peristomium and occur between the setæ tufts of every succeeding segment, except that they are occasionally absent from XXXI or XXXII. In shape they are elliptical with the long axis vertical. Ventral sensory pits appear on IV and continue without exception to XV in the position indicated above. At first very small, they increase rapidly until they exceed the lateral organs, unlike which they are always circular.

Small notopodial and neuropodial tufts of setæ occur on all somites from II caudad at the junction of the second and third rings. Both tufts are retractile into pits and the notopodial setæ are somewhat longer than the neuropodial. All setæ are very slender, flexible and thread-like, of various lengths in each tuft, and have each margin provided with a fringe of appressed hairs.

Many of the specimens are enclosed in a very tough mucous membrane more or less coated with silt, and often inhabited by small nematodes.

This species bears much resemblance to *Travisia olens* Ehlers, which has only thirty segments and rather distinct parapodial papillæ.

It is apparently an abundant worm, conspicuous from its large size, and widespread on muddy bottoms. Specimens were collected from the following stations: 4,192, Gulf of Georgia, 18 to 23 fathoms, green mud and fine sand; 4,194, Gulf of Georgia (type locality), 111 to 170 fathoms, soft green mud; 4,197, Gulf of Georgia, 31 to 90 fathoms, sticky green mud and fine sand; 4,230, Behm Canal, 108 to 240 fathoms, rocky; 4,235, Behm Canal, 130 to 193 fathoms, gray mud; 4,237, Behm Canal, 192 fathoms, green mud; 4,246, Kasaan Bay, Prince of Wales Island, 101 to 123 fathoms, gray and green mud, coarse sand and shells.

Brada pilosa sp. nov. Plate X, figs. 14–17.

This well-marked species is represented by about a dozen specimens varying in length from 15 to 30 mm., the largest having a maximum diameter of 5 mm. With the prostomium and tentacles retracted, which is the condition of all of the specimens, the form of the body is slender clavate, bluntly rounded at the anterior end and gradually thickening to about XI or XII, from which point it tapers into the rather slender, gracefully formed caudal region.

The exact form of the prostomium and mouth is not apparent, inasmuch as this region is retracted, leaving a conspicuous trifid opening which has been sometimes indicated in descriptions of other species as the true mouth. In this condition the peristomium is trilobate. All of the segments are simple rings, separated from one another by clearly defined but not conspicuous intersegmental furrows which become more distinct posteriorly. The segments pass regularly into one another without any conspicuous breaks in contour, and increase in length, as they do in diameter, to about XII, then undergo little change to the middle of the body, behind which they become again gradually shorter. Toward the posterior end they diminish to a minute pygidium which contains a small, vertical, slit-like anus, but appears to lack cirri or other appendages.

The number of segments varies from 31 to 33, the latter number being present in the type. All, including the peristomium, bear both notopodial and neuropodial setæ in tufts upon minute tubercles.

The chief characteristic of the species is the richness of its papillation. The entire dorsal surface is thickly covered with filiform papillæ especially numerous toward the ends, where they are so densely arranged that they actually touch and crowd one another. On the middle segments they are more widely separated, but are still so numerous that where *Brada villosa* bears 3 or 4 in the length of a somite, this species bears 8 to 12. They are not disposed in regular rows, but are arranged more or less irregularly at nearly equal intervals in all directions. The intersegmental furrows lack papillæ and appear as smooth lines, like narrow avenues through a grass field. Just anterior to each parapodium the papillæ become few or nearly disappear, leaving a wider open space at this point. Those papillæ remaining in this region are collected about the setigerous tubercles, but instead of becoming enlarged and forming rosettes they are actually smaller than the dorsal ones.

All of the papillæ (fig. 14) are slender, elongated and filiform with a small terminal knob. Many of them collect a thick girdle of sediment in a zone near the base, which gives the appearance of a bulbous enlargement. They differ considerably in length and toward the anterior end of the body exceed the length of the segment bearing them, so that this region has the appearance of being thickly coated with fine hairs. At the level of the ventral margin of the neuropodial tubercles the dorsal papillation ceases abruptly, and is replaced by the much smaller papillæ (fig. 15) which cover this surface. Although their number is nearly as great, these papillæ are so much smaller than those found on the dorsal surface that to the naked eye they appear merely as a fine granulation.

Every somite, including the peristomium, bears both notopodial and neuropodial setæ in small lateral tufts. The setæ of both fascicles have the same character, all being slender and transversely jointed, except at the acutely pointed tip. The former (fig. 16) are very slender and delicate and the internodes increase in length toward the tip. The latter (fig. 17) are more deeply colored, much stouter, distinctly curved and the much shorter joints decrease in length toward the tip. In both tufts the number of setæ is small, about eight notopodials and eight or ten neuropodials being the rule. On the peristomium the notopodials are very long, equalling four or five segments, and they project forward far beyond the mouth. On succeeding segments they project outward and upward and decrease in length until they are about equal to the segment bearing them. The neuropodials are rudimentary on I, but increase in both length and thickness on succeeding anterior segments, those at the posterior end becoming again more slender but without diminution in length.

As stated above the prostomium is in all cases retracted. A dissection shows that the tentacles are fine and very numerous, numbering upwards of thirty on each side. They are borne on a pair of bosses which are about twice as wide dorsally as ventrally, where they curve around the mouth and nearly meet. The palpi are very short and broad and marked by a longitudinal groove and transverse wrinkles on the ventral side, the dorsal surface being smooth. The skin is gray, but the papillæ impart to the dorsal surface a buffy yellow color.

Brada pilosa very closely resembles *Trophonia hirsuta* Theel, but is distinguished by the reduction in size and number of the papillæ forming the setal rosettes and by the unjointed tip and other peculiarities of the setæ.

The species is not uncommon northward. Examples occur from the following stations: Station 4,251 (type locality), Stephens Passage, 198 fathoms, rocky bottom; 4,235, Yes Bay, Behm Canal, 130 to 193 fathoms, gray mud; 4,252, Stephens Passage, 198 to 201 fathoms, gray mud; 4,258, Lynn Canal, 300 to 313 fathoms, mud.

Maldane similis sp. nov. Plate XI, figs. 26–30.

The type and largest example is 56 mm. long and 2.5 mm. wide, the latter being nearly constant throughout the entire length.

This species belongs to the *M. biceps* group in having the cephalic and caudal plates of nearly the same form and size. The cephalic plate is very broadly elliptical in outline, the margin little limbate, the posterior two-fifths separated by a deep notch on each side from the anterior three-fifths, the former erect and with its margin finely denticulated with from twenty to twenty-five teeth. One-fourth of the remaining margin anterior to the notch forms on each side a rather thick, narrow, spreading rim, the margin of which is quite entire or merely slightly crenulated, not conspicuously toothed as in *M. biceps*. Anteriorly the cephalic rim is separated from the postero-lateral margins of the palpode by a pair of distinct radial furrows. The palpode is very large and contributes easily three-tenths of the entire margin. It is very broad and flat, with a smoothly curved anterior border and rather more than one-third of the middle of its posterior border produced backward on to the head plate as a slightly elevated median welt scarcely rising to the height of a ridge. On each side of this, forming its lateral boundaries and the posterior boundary of the lateral portions of the palpode are the deep sensory slits, U-shaped or hooked, with the lateral limb the shorter. No furrows or other markings occur on the surface of the cephalic plate.

The mouth is large and the lips prominent and pouting. A short distance behind it is a transverse groove encircling the ventral half of the peristomium and joining a conspicuous longitudinal groove which begins at the lateral notch in the cephalic margin and passes along the side of the peristomium, to end posteriorly in the circular furrow which separates a complete narrow ring from the hinder part of the segment. Inasmuch as the posterior part of the peristomium is retracted within the anterior margin of II, this ring is completely concealed ventrally by the prominent half-collar developed in that position on the latter segment.

The next segment (II) is very short, its length not more than one-half of its diameter; the posterior third is completely separated as a distinct ring; and the anterior margin is somewhat produced into a collar, the ventral half of which springs into especial prominence abruptly at the level of the setæ. Somite III is about one-third longer than II and similarly biannulated, but its anterior margin is not collared. Thus far the skin is entirely glandularly thickened.

The following six segments (IV to IX) are decidedly longer, about equalling their own diameter, and the secondary furrow, while always present, is in a more anterior position not far behind the middle. While the three anterior segments are glandular throughout, the ones under discussion have the glandular area confined to the ventral half of the body and especially to the anterior ring in the vicinity of the tori. Somites IX and X are indistinguishable, and on the latter the now prominent tori have shifted to the posterior end of the segment.

From this point the segments continue to lengthen to XIV, which is about three times as long as thick, after which they again decrease. Throughout the middle region the integuments are soft and translucent, except for the swollen, oval, glandular areas surrounding each torus. Somites XVIII, XIX and XX are again much thicker than long and the tori are correspondingly large and prominent; XX is about one-fourth as long as thick with a prominent pair of achætous tori much below the usual level and meeting ventrally.

The pygidium consists of a very short basal ring bearing a pair of coalesced glandular thickenings corresponding to the tori of XX. The anus is conspicuous and in a dorsal position at the base of the dorsal membrane. The limbate margin of the pygidium bears a remarkable resemblance to the cephalic plate, but is oblique in a reverse direction. Its ventral two-fifths are separated rom the dorsal three-fifths by a deep rounded notch, with thickened margins which nearly meet externally and constrict its opening. The ventral portion has its margin marked by four very broad, shallow crenulations and a pair of prominent triangular lateral lobes. The dorsal plate is more prominent and flaring, with a smooth and regular margin marked only by a broad and extremely shallow median emargination.

Somites I and II and the pygidium are achætous; II bears strictly lateral setæ only, arranged in a vertical tuft just above the dorsal ends of the ventral collar; III and IV bear similar setæ tufts and very short series of uncini disposed in the same plane and both strictly lateral and sessile. On succeeding segments the setæ tufts have short, slightly oblique bases placed a little in advance of the uncini, which form lines five or six times as long as those on the preceding somites, more ventral in position, and elevated upon distinct tori. Proceeding caudad the uncinial lines increase slightly in length and become more ventral in position. The number of uncini in a torus is about 8 on III, 13 on V, 28 on X, 32 on XV and 35 on XIX.

The small tuft on II contains setæ of two kinds, the one small with a rather wide wing and abruptly tapered stem terminating in a slender tip; the other very much longer, with the stem conspicuously striated the tips rigid and less slender and the wing very narrow (fig. 26). On following somites the number of setæ increases to 10 or 12 pairs, the larger ones become stouter and the smaller more slender. By somite XV the number is further reduced; the small setæ exhibit only minute pointed tips and the ends of the larger ones are elongated and provided with ensheathing awns which appear to be disposed in symmetrical pairs.

Anterior uncini (on III) (fig. 27) have the head little enlarged and somewhat thrown back, the stout, blunt, nearly straight beak somewhat elevated, the crest composed of a single large tooth with a cluster of small ones surrounding its base, and the guard rudimentary or absent (fig. 28). In a succeeding tori there is a gradual transition to the typical form which appears at about VI. Such uncini (fig. 29) have a distinct shoulder, well-defined neck and head, and a stout, tapering, hooked beak with an acute, slightly recurved tip. The crest is well developed and formed of two transverse rows of numerous teeth which are largest at the vertex and become rapidly reduced laterally. The guard is strong and arising well below the beak sweeps boldly beyond and above its tip (fig. 30).

The type and one other specimen were taken at Station 4,264, off Freshwater Bay, Chatham Strait, 282 to 293 fathoms, on a bottom of green mud.

Maldanella robusta sp. nov. Plate XI, figs. 31, 32.

No complete specimen of this species occurs in the collection, but fortunately there are several heads and one posterior end and it is possible to so fit the fragments together as to secure a complete description.

The worm thus reconstructed is a fine large one, measuring about 195 mm. long and 7.5 mm. in diameter at the middle part. Excluding the pygidium there is twenty-one segments, of which the peristomium and first preanal segment are achætous, II bears setæ only, XX setæ only on one side, and III to XIX inclusive both setæ and uncini.

The prostomium and peristomium are completely coalesced and exhibit no trace of a dividing furrow or suture. The cephalic plate meets the dorsal profile of the peristomium at an angle of approximately 135° to 150°, so that the ventral length of the head is nearly or quite twice the dorsal. The cephalic plate has a nearly regular ellipsoidal outline, with a length of about twice the width. Its thin margin is elevated all round, highest and most erect behind, thence slightly diminishing in height to a point anterior to the middle where it is folded and sometimes slightly notched. Anterior to this point it again becomes higher and more flaring until it curves into the base of the palpode, from which it is separated by a slight cleft. Except for a faint crenulation, the margin is entire. The median ridge is rather broad and low, but extends through the anterior half or less only of the cephalic plate, ending abruptly behind; anteriorly it widens slightly and passes partly into the marginal rim, partly into the palpode. On each side of the ridge are deep sensory slits which anteriorly bend somewhat sharply outward and backward, and continue along the base of the lateral fold nearly as far as the posterior end of the median limb.

From near the posterior end of the median ridge to the lateral fold or notch in the marginal membrane passes a strictly transverse furrow on each side, leaving an extensive area in the posterior region of the head marked only by a few crescentic furrows parallel with the posterior margin. The palpode is a short, broadly rounded, rather thick, tongue-like structure which is continued backward on the ventral side as a broad welt to the mouth, within which it bifurcates to form a pair of ridges separated by a deep cleft. The mouth is relatively small and bounded by a nearly circular fold, elevated and furrowed somewhat like a piece of rope and which is open only anteriorly to admit the posterior extension of the palpode.

As before mentioned there is absolutely no visible line of separation between prostomium and peristomium, but the two together constitute a continuous head, shaped somewhat like a horse's hoof; that is, it spreads anteriorly, where it is truncated obliquely by the cephalic plate. The seven somites next following are cylindrical, with a nearly uniform diameter about equalling the posterior diameter of the peristomium, and a length but little greater. All have traces of anterior collars, which are best developed on IV to VII, on which also the glandular layer of the skin is thick and extensive. The skin of the head and somites II to IV is very smooth, iridescent and marked by fine furrows crossing in various directions, like those on the human skin. Somites V to VIII are of a dull, opaque, non-iridescent white. Following this the body is distinctly depressed and the segments elongated to two or three times their diameter. They bear prominent tori situated along broad elevated longitudinal muscle bands. While the greater part of the surface of the segments is smooth and has a conspicuous bluish iridescence, the longitudinal muscle ridges are vertically furrowed and, when the segments are much contracted, these furrows are extended nearly around the segment. With the exception of $\frac{\text{IX}}{\text{X}}$, the segments bounding which are continuous and the place of transition of the parapodia from an anterior to a posterior position, all of the furrows are well developed. Somite IX is the last exhibiting a distinct glandular region, which is confined to a narrow anterior zone. For nearly the entire length of the worm there appears in the median ventral line a neural cleft in the muscles, having the aspect of a clear, translucent, bluish line. There is no diminution in the length of the posterior setigerous segments, but the achætous segments are considerably reduced in both length and diameter. They are terete, about twice as long as thick, have the surface deeply wrinkled transversely and bear rather prominent tori in the posterior one-third. Following these is the campanulate pygidium which has a narrow ringed base and a deep cup-shaped body, the margin of which is divided into thirty-nine very regular bluntly rounded teeth, the four ventralmost of which are considerably broader than the others, while at three other points one of the latter has been replaced by two smaller ones. Both without and within the surface of the cup is longitudinally fluted, and in the deeper part of the interior numerous fine ribs, usually two to each marginal tooth, pass to the margin of the very large anus.

Somite II bears capillary setæ only, which arise as a narrow vertical tuft from a slit-like cleft into which they are retractile. Succeeding segments, to XIX inclusive, bear both setæ and uncini which are strictly lateral in position, the dorsal interval between the setæ scarcely exceeding the ventral interval between the uncini. As far as somite VIII the setæ continue to occur in the form of vertical tufts retractile within slit-like pockets; and the uncini, which begin immediately below the setæ and lie in the same plane, form strictly linear series sessile or even depressed below the surface. On IX and all subsequent segments the setæ are situated on rather prominent wart-like papillæ in the form of crescentic tufts open below; and the uncini are elevated on the crests of swollen tori. On II, III and IV the parapodia are situated in the anterior one-third; on V and VI they are but little anterior to the middle; on VII, VIII and IX they are again near the anterior end; on X they shift abruptly to the posterior one-third and so remain to the last. The posterior tori are especially prominent. Whether XX is normally setigerous cannot of course be determined until additional specimens are known.

The number of uncini increases toward the posterior end, the counts being 22 to 26 on III, 35 to 42 on V, 45 to 50 on X, 53 to 57 on XV and 60 on XIX.

The setæ are numerous and form dense tufts. On II those of one series are shorter and about three times as thick as the others and have well-developed wings. Those in the other series are slender and nearly wingless. On succeeding somites all of the setæ become much more elongated and more slender, but do not differ otherwise, and apparently lack altogether any lateral hairs or awns.

As indicated above uncini (fig. 31) are numerous on all segments;

and are stout, striated, and of a deep yellow color throughout. They differ very little in form on the different segments, the only noticeable distinction being that the extreme anterior ones have the crest teeth less well developed and the guard hairs fewer. The rather long, slender, curved stem has a distinct but tapering shoulder, a rather long, erect neck and an enlarged head, below which is a very prominent square guard process (fig. 32). The beak is stout and hooked, the crest teeth three or four, the lowermost very large, the upper small or obsolete; sometimes, especially on anterior uncini, a pair of small but distinct lateral teeth is present. The guard is strong and consists of about sixteen stiff hairs which arise from a scale-like base ensheathing the front of the guard process, and spread regularly in an even curve around the end of the beak, above which they arise convergingly to a considerable height.

The body of the alcoholics is generally colorless or pale yellow, but the cuticle has a strong bluish iridescence throughout.

A portion of a tube is soft and flexible, consisting of a thick mucoid substance covered with a stratum of moderate thickness of soft grayish brown silt.

This species is evidently related to the three species of *Maldanella* described by McIntosh from the deep waters of the oceans of the Southern hemisphere. The Japanese maldanid *Clymene harai* Izuka (=*Axiothea campanulata* Moore) also belongs to this genus.

The sources of the examples of *M. robusta* are Station 4,197, Gulf of Georgia, 31 to 90 fathoms, bottom of sticky green mud and fine sand; Station 4,230, Behm Canal, 108 to 240 fathoms, rocky bottom; and Station 4,246, the type locality, Kasaan Bay, Prince of Wales Island, 101 to 123 fathoms, bottom of green mud with coarse sand and shell fragments.

Clymenella tentaculata sp. nov. Plate XI, figs. 33–35.

This very interesting species is unfortunately imperfectly known, the following description being based upon one anterior and one posterior piece which may be parts of the same individual and which together represent nearly an entire worm.

The former measures 22 mm. long and 2 mm. wide at IX and consists of the prostomium and nine segments; the latter is much twisted and the five setigerous segments, four achætous preanal segments and pygidium measure about 20 mm. long.

The cephalic plate is very much expanded and flares widely at the margin. Its outline is very broadly oval. The hinder third of the rim is separated from the anterior two-thirds by a pair of small lateral incisions, behind which it is more erect and diminishes in height to a minute median posterior notch. Anterior to the lateral incisions the rather abruptly widened and flaring margins continue undiminished almost to the palpode which they join on each side. The cephalic margin is everywhere smooth and its margin entire. A pair of conspicuous sensory slits divide the central disk of the head for about the anterior five-sixths of its length into three narrow longitudinal areas of equal width which are united behind. The central one is somewhat ridged and widens almost imperceptibly as it passes into the palpode anteriorly. The palpode consists of a short rounded base bearing a slender, elongated finger-like process on its median anterior margin.

The cephalic plate forms a dorsal angle of about 120° or less with the peristomium, which is indistinguishably coalesced with the prostomium. It is little more than one-half as long as the cephalic plate and its surface is slightly granulated but unwrinkled. Owing to the protrusion of the proboscis, which has a depressed acorn-shape, with the basal division thickly papillated, the mouth is invisible.

Somite II (the first setigerous) has a length about equalling the width at the anterior end, from which it gradually diminishes in diameter caudad. The next two segments are narrowerer, after which the diameter increases gradually to VIII, though the length remains nearly constant; IX has the same diameter, but if complete is scarcely half as long as VIII. All of these segments are transversely wrinkled superficially and are provided with a distinct, raised neural line. No prominent collars but merely a low free rim, most distinct on V, are developed on their anterior ends.

Owing to the much coiled and twisted condition the real proportions of the distorted posterior segments cannot be easily ascertained. They are evidently three or four times as long as wide, slender and thin-skinned, except posteriorly where the prominent, swollen and glandular parapodia are developed. There are no especially developed glandular zones or muscular ridges and the neural line is elevated throughout. The last six or seven segments decrease in length and the entire region tapers to the pygidium. The first of the posterior achætous segments is about one and one-half times as long as wide and of a shape similar to those preceding it, having fully developed but naked tori on the posterior end. The next three are simple rings without tori and of rapidly decreasing length. Terminating the body is a remarkably small, top-shaped pygidium lacking any limbate margin. Instead there arises around the base of the anal papilla, which constitutes its greater part, a circle of twenty-three separate and distinct cirri, all of which are slender, regular and equal, and not, as in many species of the genus, alternately longer and shorter. Apparently they increase slightly in size from the dorsal to the ventral side; and the median ventral one is much elongated, its length equalling that of the four achætous segments combined or about ten times the length of the other marginal papillæ, and it is very slender distally.

On the first three setigerous somites (II to IV) about thirty setæ occur in each group and are disposed in small vertical tufts just above the lateral line and about one-third of the length of the somite from its anterior end. The small number of uncini (3 to 5) are sessile in short, transverse lines just below the setæ. On succeeding segments the setæ are more numerous and project upward and outward in tufts from small tubercles. The uncini are more numerous (13 on V, 20 on IX, and still more posteriorly) and form longer lines widely separated below by a space of twice or more their own length. The tori have become prominent swellings. On IX they have become transferred to the posterior end, although the boundary between this segment and VIII is not clearly defined. This condition of the tori continues throughout the body, though they become even more prominent posteriorly and are united across the dorsum of each segment by a glandular band. The dorsal interval between the setæ is about equal to the ventral interval between the uncini. The first achætous segment at the posterior end bears a pair of perfectly normal tori, but no setæ or uncini.

The setæ are often imperfect and their distribution is worked out only incompletely. Anterior segments have them all slightly curved, delicate and narrowly winged, with very slender, tapering tips. They occur in two series, one of finer, the other of coarser setæ. Farther back these two kinds become further differentiated. Both become longer and the slender ones provided with short basal wings, beyond which is a delicate capillary tip doubly fringed with strongly divergent, very fine hairs.

All uncini are yellow and have the stems longitudinally striated. Those of somites II to IV, in which the number is small, have the form shown in fig. 33. The stems are slender, slightly curved, regularly enlarged, but with no distinct nodulus, then slightly constricted to a neck, bearing a scarcely enlarged head (fig. 34), with a simple, tapering unhooked beak, a small crest of three minute teeth and apparently no guards. These uncini increase regularly to the dorsalmost, which also has by far the most prominent beak. On the remaining somites the uncini differ decidedly in the form of the head (fig. 35) which is much enlarged backward. The smaller beak is more curved and hooked, the crest high and prominent, with five or sometimes six non-fibrous, imbricated teeth of diminishing size. The guard consists of several (about 8) fine tapering hairs, which arise from a plate just beneath the beak, the inferior outline of which they follow to the tip, above which they then rise as curled inarched filaments.

The alcoholic specimen is colorless, but the greatly developed parapodial plexuses of bloodvessels indicate red bands during life.

Some fragments of tubes are 3 mm. in diameter. Their flexible walls are composed of a soft mucoid membrane covered with a thin coating of very fine neatly deposited sand.

This species presents interesting resemblances to *Praxilla gracilis* (Sars) Malmgren in the form of the head and tentaculiform palpode, to *Clymenella catenata* (Malmgren) in the number of achætous preanal somites, and to *C. rubrocincta* Johnson in the elongated median ventral pygidial cirrus.

It is known only from Station 4,264, off Freshwater Bay, Chatham Strait, July 25, 1903, 282 to 293 fathoms, bottom of green mud.

Nicomache carinata sp. nov. Plate XI, figs 36-39, and Plate XII, figs. 43 and 44.

A well-preserved but somewhat contracted specimen (the type) is 65 mm. long and 3 mm. in diameter at the thickest part. Another incomplete but more fully extended example must have exceeded twice this length when complete.

The prostomium and peristomium are coalesced. forming a continuous head about 1¾ times as long as wide. The prostomial region is nearly vertical with about its medial ⅓ formed by a ridge, which forms a prominent profile, somewhat arched above and ending below and anteriorly in a transverse crescent, separated by a slight groove from the short, somewhat thickened palpode. The latter is continuous with the similarly thickened lateral margins of the head, within which is a pair of shallow longitudinal depressions, while within these again and close to the median ridge are the distinct longitudinal sensory slits or nuchal organs.

The dorsal peristomial region is somewhat tumid anteriorly, with a nearly straight profile posteriorly, but strongly arched transversely throughout. From the prostomium it is slightly distinguished by a faint transverse groove which passes laterally into the depression mentioned above and the groove which continues the latter posteriorly to the end of this segment. The mouth is a large elliptical opening surrounded by a rugous, furrowed lip. Just behind this lip is a shallow transverse ventral groove, while near its posterior end this segment is completely encircled by another faint furrow which, like the groove mentioned, is met by the longitudinal furrow on each side.

Besides the pygidium the type has 24 segments, while the only other complete specimen in the collection has 25. The peristomium is achætous, and somites II to XXII (or XXIII) inclusive are setigerous, leaving two preanal achætous segments, as in *N. lumbricalis*. Somite II is as long as the head; the next seven or eight segments are successively of slightly increased length, the last named being nearly twice as long as the first. Behind IX several segments remain nearly equal in length, then the length diminishes, at first slowly, then rapidly to the last, the last three setigerous segments each equalling III in length and the two achætous preanal segments together barely exceeding the last setigerous. Somites II, III and IV are of much greater diameter anteriorly, and slope to the posterior end which is inserted slightly into the next succeeding segment. The next five segments (V to IX) are more nearly cylindrical, but bear slightly produced glandular collars at their anterior ends. The last four are simple short rings. At the two ends of the body the intersegmental furrows are deep and distinct, but in the middle region they are obscure.

The pygidium is funnel-form, but little widened, regular and somewhat longer than the last two segments combined. On the type its margin is divided into twenty-one equal and regular triangular teeth each with a minute cirriform tip. The only other anal funnel present has but sixteen marginal teeth and cirri. The anus is central and very large, and is surrounded by about ten regular lobes separated by as many radiating furrows. At about somite VIII the neural groove begins to be distinct between the lateral muscle ridges and continues to XXIV, from which point a faint neural line continues across the pygidium to end in the median marginal tooth which is narrower than the others. The other anal funnel has the three median ventral teeth smaller and the nerve cord terminating between two of them.

No distinctly elevated tori or setigerous tubercles exist anterior to XIII, on which the latter are midway between dorsum and venter, and the ridge-like tori extend from just below them through the centre of the broad glandular zone nearly to the neural line. On succeeding segments they are even more prominent, and behind XV have shifted with the glandular zone to the posterior end of the segment. Toward the posterior end as the thin-walled portion of the segment diminishes in length they become more central, and finally on XXI and XXII the swollen glandular tori form the entire length of the segment and bear the uncini along the middle. Although XXII and XXIV are achætous they are similarly constituted.

The head and first four segments are very smooth, iridescent and of a uniform deep purple color above. The next three segments are smooth and dull yellowish throughout; those of the middle region have pale anterior glandular zones, the rest being yellowish and roughened, while posterior segments are pale throughout and thin-walled, except for the ventral longitudinal bands and posterior glandular zones.

The peristomium and two preanal segments are achætous. Somites II, III and IV have a single stout spine situated at about the end of the anterior one-third of the segment and about twice as far from the dorsal as from the ventral median line. A little above and in front of this is a short vertical series of capillary setæ. On the remaining segments the setæ form more prominent tufts arranged in vertical series midway on the sides of the body and in the middle of the glandular area which lies on the anterior end of the somites as far as about XV, then for some segments on the posterior end, and finally, with the disappearance of the non-glandular region, occupies the entire segment. Uncini occur on all segments from V to XXII inclusive. They are in all cases arranged in a single series in the same plane as the setæ, and extend in a line, for a greater or less distance according to their number, on to the ventral surface. On the anterior segments they are slightly larger and fewer, the number counted in the type being 7 to 9 on V, 16 to 19 on X and 10 to 12 on XXII.

The single ventral spines on II, III and IV are stout, fibrous and yellow, tapering to a slightly curved, blunt, hard point. On the same segments the notopodial tufts contain about six longer, stouter setæ (fig. 44) with longitudinally striated stems tapering to stiff, straight, acute tips and provided with a broad and extensive wing on one side and a very narrow one on the other; also a corresponding number, arranged in a parallel row, of much shorter, more delicate setæ (fig. 43) with nearly wingless stems tapering to a long hair-like and very delicate, flexible tip provided on each side with minute cilia-like hairs. The latter are so minute that they are visible under high powers only. Except for a slight increase in number the stouter setæ remain unchanged throughout, but the more delicate ones become considerably modified. By about the tenth segment they have become more numerous; some of them (fig. 43) are only moderately elongated (about as much or only slightly more than those of the preceding segments), but the lateral processes have become much larger, ensheathing and awn-like, and may be arranged spirally (fig. 43*a*); others are very greatly elongated, thread-like, openly spiral, tapered very gently to acute tips, and have the lateral processes reduced to minute appressed scales (fig. 36). The latter spring in a group from the ventral side of the bundle.

Typical crochets (figs. 38 and 39) have rather slender, strongly curved stems with an asymmetrical nodulus near the middle, the head very little enlarged and provided with a stout, rather long, moderately acute, strongly hooked, and slightly recurved rostrum, above which is a prominent crest consisting of five, or sometimes of four, depressed, overlapping, diminishing teeth. There are no lateral teeth, but the striations end in several groups of conspicuous markings just below the principal teeth. The guard arises close beneath the rostrum, but separated from the latter by a distinct space. There is no distinct guard process or shoulder, but the guard arises as a distinct transverse plate, soon becoming divided into about twelve slender, tapering filaments, which spread around the end of the rostrum or overarch it. The stem, neck and posterior part of the head are strongly striated. Posteriorly the stems of the uncini increase in length. On somite V all of the uncini have the form shown in fig. 37. The stem is straighter, beak less hooked and the teeth of the crest fewer and more erect. The guard is rudimentary.

The head and succeeding three or four segments are very smooth, iridescent and of a uniform deep purple or reddish-brown color above, not at all spotted or blotched; the next three segments are smooth and yellowish throughout; those of the middle region have pale anterior glandular zones, the remaining parts being yellowish and roughened; while posterior segments are pale throughout and thin-walled, except in the position of the longitudinal muscles and glandular zones.

The tubes are stout, thick-walled, hard but fragile structures, composed of fine sand grains, sponge spicules and bits of rock cemented together firmly and lined by a thin mucoid layer. Several tubes are sometimes coherent side by side.

Fragments of this species occur in the collections from the Gulf of Georgia, Station 4,197, 31 to 90 fathoms, on a bottom of sticky green mud and fine sand, and Station 4,198, 157 to 230 fathoms, on a soft green mud bottom. The type locality is Station 4,227, in the vicinity of Naha Bay, Behm Canal, 62 to 65 fathoms, dark green mud and fine sand.

Nicomache carinata is easily distinguished from *N. personata* Johnson by the possession of two achætous preanal segments, while the latter has but one. In this respect it resembles *N. lumbricales* Malmg., but differs from that species in color, the form of the uncini and spines and the greater number of marginal divisions of the pygidium.

Lumbriclymene pacifica sp. nov. Plate XII, figs. 40–42.

The type and largest complete specimen, which is unduly elongated through maceration of the middle region, is 103 mm. long and 2 mm. in diameter.

The head (composed of prostomium and peristomium) is slightly compressed laterally and very short, the length only very slightly exceeding the depth. It totally lacks a cephalic plate and has a nearly straight dorsal profile, meeting the vertical and convex anterior profile at nearly a right angle. There is just the faintest indication of a palpode and of the anterior end of the median ridge. About midway on the side of the head a longitudinal furrow extends from the furrow $\frac{I}{II}$ to a point just over the mouth, and just anterior to the ends of these furrows are the nuchal organs—a pair of small round depressions. A few faint short furrows run from near the end of the longitudinal furrow toward the mouth, and behind the latter the longitudinal furrow is cut by a transverse furrow which is very strongly developed on the dorsal side but fades out ventrally. From the posterior end of the peristomium a short re-entering half-ring is cut off ventrally by a rather distinct furrow which ends dorsally at the longitudinal furrow. The mouth is a rather conspicuous transverse slit situated about opposite the middle of the head and bounded both anteriorly and posteriorly by rather prominent lips, the former of which is marked by a short but deep longitudinal furrow.

Somite II is slightly shorter than the head. Behind it the segments increase regularly in length to IX at least, which is about three times as long as the head. Behind this several segments are probably still longer, but owing to their much softened state the exact length is uncertain. At the posterior end XVI is about as long as VI or VII and succeeding segments diminish rapidly, XIX and XX together only about equalling V in length. The two achætous preanal segments are again much reduced.

Except that at the extreme posterior end a few are provided with prominent tori, all somites appear to be perfectly cylindrical. Somites II, III and IV bear setæ about the middle and just behind them is a faint furrow. The next five bear them near the anterior end just behind the glandular girdle. Posterior somites have their large swollen tori situated near their posterior ends and bearing the small tufts of setæ above and moderately long lines of crochets below. The two preanal segments have two tubercles on each side, one above the other, in the position of the tori, but without setæ or uncini.

In the type the pygidium is very short—less than the two preanal segments—and is terminated squarely by a simple slightly convex plate of broadly elliptical outline. Another specimen has the pygidium more extended and nearly twice as long (on the dorsal side) as the two preceding segments, and the terminal plate is oblique at about 45°. In both specimens the plate is margined by a just evident fold, continuous except on the median ventral region. Just anterior to this margin dorsally is the anus.

The color is well preserved at the anterior end in a sharply contrasted pattern of reddish brown and white. On the head the former color occurs as follows: over the entire frontal surface, a half-ring on the dorsum of the posterior end of the prostomium, and a broad ring which occupies most of the peristomium and which is much more deeply colored dorsal to the longitudinal furrow than below it. The white areas are a broad band occupying the sides and dorsum of the prostomium above the mouth, and a very narrow ring on the anterior end of the peristomium. On several succeeding segments the arrangement is in a narrow anterior red ring, then a white ring occupying most of the glandular zone, and succeeding this an extensive red area occupying all of the rest of the segment. These colors are best developed on the dorsum, and gradually fade until by about somite VII only a nearly uniform dull yellow prevails. The glandular areas are, however, always whiter and more opaque than the remainder of the segment.

The peristomium and two preanal somites are achætous, II to V bear small tufts of dorsal capillary bristles, and immediately below them two stout straight spines. Remaining somites have dorsal setæ tufts, and below them ventral series of crochets occupying from one-eighth to one-sixth of the circumference of the segment.

The anterior spines (fig. 40) are deep yellow, with opaque fibrous centres and straight blunt ends. In each tuft the setæ number from fourteen to twenty in two ranks. On the proximal part of the exposed portion is a rather wide wing, obliquely striated and often frayed and fringed on the free margin. Just below this the shaft is usually constricted, and beyond it tapers to a capillary tip which is short on the anterior and very long and delicate on the posterior segments, but always, so far as determined, quite devoid of hairs or awns.

Crochets are always few in number, never more than nine to fourteen occurring in each torus. They (fig. 41) are similar on all segments and have well-differentiated shoulder, neck and head. The latter (fig. 42) bears a stout, strongly hooked beak, above which are four stout profile teeth of diminishing size, flanked by a few small lateral teeth. The guard arises well below the beak and consists of about twelve coarse fibres which spread in front of and above the apex of the latter. The internal fibrous structure is strongly developed.

Several tubes of this species occur in the collection and are interesting in structure. They measure 70 to 80 mm. long and 2.5 mm. in diameter, and occur either singly or attached in groups or to foreign bodies. Their walls are thin but hard and very brittle and are composed chiefly of small sand grains and sponge spicules, the latter of which are so attached that their pointed ends project freely toward the mouth of the tube. Various kinds of foraminifera and other foreign bodies are attached to the tubes, which are always dark-colored at the distal end and clean and pale elsewhere.

Two complete worms and a fragment together with four or five tubes were taken at Station 4,264, off Freshwater Bay, Chatham Strait, 282 to 293 fathoms, bottom of green mud.

Sabellaria cementarium sp. nov. Plate XII, figs. 45–51.

The fine species which represents the genus *Sabellaria* along the Pacific coast from Washington to Alaska is represented by a number of specimens, but unfortunately only one of these is complete, the others having lost the posterior end either through an attempt to remove the living worm from their tubes or by maceration in the tubes.

The type and only perfect specimen is 81 mm. long, of which the very slender fecal tube contributes 28 mm. The operculum has a diameter of 4.5 mm., the thorax a width of 6 mm., from which thickness the abdomen tapers regularly to about 2 mm. at the posterior end and then suddenly contracts to the 1 to 1.5 mm. of the fecal tube.

Counting the peristomium there are five thoracic segments exhibiting three distinct types of setation, then follow forty ordinary abdominal segments and about forty-six segments in the reduced fecal tube; finally the pygidium is a tubular structure 1.5 mm. long and .6 mm. in diameter with its posterior end serrate with about twenty minute teeth.

The prostomium is minute and completely concealed beneath the enormously developed peristomium. The small slit-like mouth is enclosed between a pair of closely appressed longitudinal folds, bounded laterally and somewhat enfolded ventrally by the enlarged palps, which are completely connate with the peristomium.

The peristomium is greatly enlarged and in the type measures 6.5

mm. long and 4.5 mm. wide. Dorsally it is completely closed, overarching and concealing the prostomium and mouth. Together with the palpi which it bears below it forms a broad flat plate, of which the palps form the margins and are rolled inward and nearly touch ventrally. At its distal end the peristomium bears the operculum—a circular or somewhat elliptical disk directed slightly dorsad and composed of three whorls of stout, stiff, hard spines or peristomial setæ, so fitted together that they form a flexible and at the same time close-fitting and impenetrable plug to the tube. There are some indications in one specimen that this region may be regenerated when lost. The paleoli of the three rows differ in form and number as indicated below. Just external to and below the operculum is a circle of rather prominent conical opercular papillæ, which probably represent the much subdivided dorsal cirri of the peristomium. They are clearly divided in two symmetrical halves like the opercular paleoli, and number 16 to 20 on each side.

The palps may be opened from the ventral side and spread, together with the peristomium, as a flattened plate deeply pigmented below and bearing the branchial folds on the lateral thirds. Anteriorly the two palps are conjoined in the operculum, the branchiæ also meeting in the middle line below and behind the operculum. From 12 to 18 of these branchial folds or ridges occur on each side, the usual number in full-grown specimens being 18 pairs. They are prominent ridges running transversely across the free ventral margin of the palps and diminishing in size from behind forward. Owing to the medial bending of the anterior end of the palps, the anterior six pairs of branchiæ are arranged in the form of an arch and several of them lie in a nearly antero-posterior plane. Each gill consists of a stiff plate or ridge, with a serrate free margin behind which the filaments are borne. Except the very last, which usually bears but 10 filaments, the posterior gill ridges have about 20, the number decreasing regularly to the most anterior. All gill filaments are very slender and thread-like and the longest posterior ones equal the width of the peristomium.

At the base of the branchial region is a pair of short rounded lobes having much the aspect of the branchial plates, but shorter and thicker than they and coming in contact across the mouth slit. Continuing from this dorsally is a sloping ridge bearing a pointed conical cirrus longer than the rounded lobe, and above this again a small tubercle carrying a fan-shaped tuft of slender setæ.

Somite II is a short, simple ring partly fused with I and III ventrally to form the first ventral gland plate. Laterally it bears three conical cirri, the ventral about as long as the segment, the middle about two-thirds as long, and the dorsal more than twice as long, slender distally and resembling the branchiæ, with which it stands as the first of a series.

The remaining three thoracic segments are a little longer and IV and V bear distinct ventral glandular plates. Their neuropodial setæ are stouter than those on I and II, but have no associated cirri and arise from the anterior margin of the segment on a level about midway between the neuropodial tufts of I and II. Each of these segments bears a dorsal cirrus or gill similar to that on II and in line with it, leaving a broad, naked area on the dorsum. Unlike II these somites bear no middle cirrus, but in place of it a prominent vertically elongated notopodial tubercle bearing a vertical series of large, coarse, paddle-shaped setæ.

Abdominal segments are somewhat flattened and only obscurely separated. As indicated above they decrease gradually in both length and diameter to the caudal appendage. Dorsally they present a somewhat arched area between the rows of gills and ventrally are excavated by a deep groove for the fecal tube, which is held in place by the tufts of slender neuropodial setæ crossing from side to side beneath it.

The body walls are exceedingly thin and delicate in the dorsal median area and permit the intestine to show through distinctly; the sides and ventral parts are decidedly thicker and more muscular. The abdominal gills have the same general form and position as the thoracic but are more perfect in structure, having larger blood-vessels, thinner walls and more numerous and prominent transverse ciliated ridges along their medial aspect. The first five or six are also much longer and have a length equal to the width of the interbranchial area. From this point they decrease in size very gradually and are totally absent from the last five or six segments.

On the first abdominal segment the parapodia are very extensively developed and the notopodial uncinigerous tori occupy the entire side of the segment from the branchia nearly to the ventral median line. Ventrally they become higher and terminate in a small free lobe, from beneath which arises a delicate ventral cirrus, and below this again the small neuropodial tubercle with its tuft of setæ. The tori decrease in length chiefly from the dorsal end, at first very slowly, then rapidly and at the same time become more sharply defined as distinct, thin, outstanding lobes bearing the uncini on their margins. Toward the posterior end they become merely small prominent processes with somewhat constricted stalks standing midway between the branchiæ and the neuropodial setæ tuft. After the first abdominal segment the neuropodial cirrus quickly becomes reduced and in four or five segments has become quite rudimentary or totally absent.

The caudal or fecal tube has already been partly described. It is very delicate and thin-walled and bears no trace whatever of parapodia. Along its ventral side, however, what appear to be nerve ganglia can be distinctly seen and counted through the body wall, and delicate lines running in pairs from their neighborhood dorsad and cephalad have the same metameric arrangement.

The type specimen is richly colored. The outer whorl of paleoli are a warm golden, the inner a golden brown. The thoracic region is a rich sienna brown, especially deep on the dorsal interbranchial region of the peristomium as well as of succeeding segments, and on the sides of the latter and the first ventral plate. The ventral post-branchial lobe of I and the dorsal branchiæ are also well colored, but other cirri are pale. The gill filaments are pale purple, each with a deep brown basal spot. This specimen is a male, and the abdomen is colored pale cream from the contained sperm. Except for delicate lines formed of minute dots of reddish brown, which begin at the ventral setæ tufts and then pass dorsad along the anterior margin of the segment and in most cases continue on to the antero-lateral margin of the gill, there is no pigment in this region. The intestine is filled with a greenish-gray matter that colors the fecal tube. Most of the other specimens are paler, but one has the anterior brown parts of a deep chocolate. The genital products escape by means of a pair of openings through the body walls behind the parapodia of each segment and in several cases from large masses within the tubes.

The opercular paleoli vary from bright yellow to the more usual deep golden brown. They are all very stout, hard and rigid, and of peculiar irregular forms difficult of accurate description. All have slender, elongated stems, smooth superficially but striated longitudinally at the core, bearing very heavy and strong external blades, the great part of whose surface is roughened by numerous fine wavy parallel ridges which are slightly imbricated with their edges directed outward. The markings are not shown in the figures.

The outer whorl of normal opercula contains from 39 to 64, according to the size of the worm, between 50 and 60 being the most usual number. They are arranged in two symmetrical groups, though there appears to be a decided tendency for the right half to include one or two more than the left. Exclusive of the stem they (fig. 45*o*) consist of a massive irregularly twisted base, from which arises a tongue-like piece the end of which is strongly flattened at right angles to the greatest thickness of the base and terminates in a fringed tip, from the central cleft of which arises a slender, densely hairy process (fig. 46). These paleoli are so arranged that they present a spreading rim, in which their twisted form causes the basal portions to be somewhat imbricated from below dorsalwards.

The middle paleoli number from 14 to 25, usually about 20, in symmetrical halves. They (fig. 45*m*) also have a thickened base, from which arises a very prominent upright spine, gracefully curved and tapered to an acute tip. The inner circle of paleoli contains from 14 to 22, but in nearly all cases they equal and alternate with those of the middle row. They (fig. 45*i*) have abruptly widened cleaver-shaped ends bearing a knife-like edge and strengthened on the opposite side by a thickened rim which ends in a short triangular beak. They are disposed somewhat obliquely to the middle line and converge ventrally.

Somite II bears a small tuft of strongly doubly-fringed, feather-like neuropodial setæ (figs. 49 and 50). On III the neuropodial setæ are partly of a short, more brush-like form and partly very small and of a slender, slightly fringed form. On the remaining thoracic segments they all become more sparsely fringed. The notopodials (fig. 47) on III, IV and V form a single vertical series of stout setæ, shaped like an Indian paddle with the end much split and frayed. At the base of each of these is a minute spatulate hairy setæ, with the end entire and somewhat produced in the middle, and the blade bent flatways.

Abdominal setæ are all very long and slender, but owing to different states of development appear of unequal length. Their shafts (fig. 48) are provided with numerous whorls of hairs united at the base into collars and much produced on one side.

The uncini (fig. 51) are very numerous and vary from 100 to 200 or even more on a torus. They are nearly colorless, delicate and inconspicuous, and consist of narrow elongated bodies provided with from 7 to 9 (seldom the former) very acute, appressed, overlapping teeth which are arranged in one series and diminish in size toward both ends. Each bears a stiff and rather brittle tendon at each end, the upper one being short and expanded distally into a small plate, while the lower bifurcates immediately into a shorter, irregularly thickened, more ventral branch and a slender uniform branch greatly elongated to many times the length of the uncini.

This species is probably rather common and occurs in the collections from the following stations: 4,220 (type), Admiralty Inlet, near Port

Townsend, Washington, 16–31 fathoms, green mud, sand and broken shells; 4,247, Prince of Wales Island, 89–114 fathoms, green mud with sand and broken shells; 4,274 (cotypes), Kadiak Island, 35–41 fathoms, green mud and fine sand.

Samytha bioculata sp. nov. Plate XII, figs. 52, 53.

The anterior prostomial lobe is quadrate, broader than long, its anterior border wider than the posterior, slightly concave and its lateral angles somewhat produced. Immediately behind and separated from it by a distinct transverse furrow is a second small quadrate lobe, with a rather prominent eye or close aggregation of several pigment specks at each antero-lateral angle. Behind this lobe the peristomium forms a broad, smooth, slightly convex area reaching to the branchiæ. Ventrally there is a very broad truncate under lip with a glandular margin. In the two known specimens only four to six very short clavate tentacles exist on each side.

The considerably enlarged peristomium projects ventrally as a broad lobe enveloping the lower lip and is marked by a narrow transverse line of gland cells. Elsewhere it is a simple smooth convex ring. The second somite is about two-thirds as long and marked by a broader glandular half-ring. The third (first setigerous) somite is very short, but succeeding ones increase in length rapidly and by about VIII equal the peristomium. All of the thoracic segments, of which there are nineteen, the last seventeen of which are setigerous, are somewhat muscularly thickened on the ventral half and provided with a narrow presetal half-girdle of glands. The entire dorsum between the setæ tufts is thin-walled and smooth throughout.

Fourteen segments in the type and larger specimen, and thirteen in the smaller cotype, form the abdomen. In the former this region comprises about one-third of the entire length; in the cotype not more than one-fourth. Owing to the projection of the parapodia from the angles this region is decidedly quadrate, but somewhat arched dorsally and marked ventrally by a narrow neural groove. The somites diminish regularly in all dimensions from before backward and the entire region tapers to the pygidium, which forms a ring surrounding the large anus and bears a pair of slender, prominently projecting cirri about equal to half the diameter of the body at the posterior end.

The four pairs of branchiæ are so much crowded antero-posteriorly that they appear to form a single series extending quite across the dorsal area of II and III. Closer study shows that two are anterior and slightly more lateral and two posterior and more median. They are all similar, slightly flattened, slender and elongated, their length being about three times the diameter of the region of the body from which they spring.

Setæ begin on III as a pair of minute tufts arising from an elevated position just beneath the gills. The tufts on IV are also small and placed nearly as high. On succeeding somites the setæ become larger and more numerous and the tufts gradually assume a low station on the sides of the body as the abdomen is approached. They continue to XIX.

Uncinial tori begin on VI, or the fourth setigerous somite. Throughout the thoracic region they are low, but freely projecting, compressed folds which arise immediately ventral and slightly posterior to the setæ tufts and end ventrally in a freely hanging lobe or process. On abdominal segments the notopodia are distinctly separated from the neuropodia and project as small papillæ from the dorsal angles of the body. The neuropodia are small, compressed lappets constricted at the base and bearing the uncini along the free margin; apparently they lack cirri.

Setæ are all of one kind, slender, tapered and narrowly winged on both sides of the free portion nearly to the very acute tip. The uncini form a close single rank in which they are quite numerous (108 on X). From dorsal to ventral end of each series they decrease in length. They (fig. 52) are roughly triangular with a nearly square but sometimes projecting upper ligament process, above which the tooth-bearing margin rises but slightly. The inferior process varies in form, but is usually more or less incurved. Those of the type specimen almost constantly bear four long, slender, acute, overlapping teeth, the ventralmost of which is the stoutest, the two middle the longest and the upper the most slender. On the cotype a fifth smaller and sometimes minute tooth exists at the upper end of nearly all uncini (fig. 52*a*). Abdominal uncini (fig. 53) have the same form, but are much fewer in number.

This species is founded on two much macerated specimens, of which the larger and type is 30 mm. long and filled with large ova. The smaller example is 16 mm. long.

A nearly complete tube is 65 mm. long. Its basal third is soft, thin-walled and membranous. Beyond this the walls gradually thicken by the accumulation of silty material until they attain a diameter of about 5 mm. This entire region of the tube is supported and protected by siliceous sponge spicules, the ends of which project in all directions and produce a very firm and bristling structure.

Both specimens come from Station 4,197, Gulf of Georgia, 31 to 90 fathoms, sticky green mud and fine sand.

Amphicteis scaphobranchiata sp. nov. Plate XII, figs. 54–61.

A single well-preserved specimen represents this noteworthy species. The length without branchiæ is 32 mm., the branchiæ 7 mm., and the maximum width in the anterior part of the thorax 2.8 mm.

The anterior lobe of the prostomium (fig. 54) is almost completely divided by a longitudinal furrow into a pair of somewhat slender tentacle-like divisions which are somewhat divergent anteriorly. A narrow transverse welt passes across the base of this region and is partly concealed by the much larger and more prominent sensory folds which lie behind it. These pass from the lateral borders of the head nearly transversely to the middle line, where they meet in a wide angle. Laterally they are continuous with the lateral lobes or folds which join the frontal lobe beneath. The tentacular membrane lies below the frontal and lateral lobes and is partly embraced by the peristomium. It bears from twelve to fifteen tentacles on each side, the lateral ones being very short and the middle ones as much as two-thirds the width of the thorax. All and especially the shorter ones are more or less clavate.

The prostomium passes without any clear demarkation into the peristomium, which reaches as a smooth unbroken convex surface to the branchial segment. Dorsally it is remarkable for its extent, smoothness and absence of furrows. Its length and breadth are each equal to about two-thirds of the width of the branchial segment. In the middle line it reaches to the interbranchial shield, but its postero-lateral angles are cut off by the paleolar tubercles and lateral portions of somite II. Ventrally the peristomium forms a large, broadly truncated lower lip, slightly inserted into II and embraced laterally by the paleolar tubercles.

The second somite is more than half as long ventrally as the peristomium, but except for the narrow strip extending dorsally beneath the branchiæ it ends abruptly at the enlarged paleolar tubercles. Somite III is very short and IV slightly longer, the two combined just equalling the length of II. Behind this region the segments again diminish in both length and diameter to the pygidium. With the exception of the last three or four, the thoracic segments are ill defined on the ventral and not at all on the dorsal side. Up to the level of the setæ tufts, where a lateral shelf-like ridge is formed, the body walls are rather thick and firm, with slight anterior glandular bands and obscure ventral plates. Dorsal to the setæ the body for the entire length is perfectly smooth and unsegmented. The last three or four thoracic segments are transitional in character to the abdominal. The latter are more distinctly differentiated, owing in part to their more prominent parapodia and in part to the greater depth of the ventral furrows. The last few segments are separated by obvious furrows even on the dorsal side. Owing to its being crushed the pygidium cannot be described further than to refer to the pair of prominent, slender and stiff cirri which it bears. A distinct neural groove extends throughout the abdominal region and even on to several of the posterior thoracic segments. A remarkable smoothness and peculiar dull iridescence characterizes the entire cuticle of this worm.

If normally formed in this specimen the branchiæ are highly characteristic. All four pairs are large and stout and arise in the usual manner, two pairs anterior and more lateral from II and III and two more posterior and median from IV, those of each side being completely coalesced at the base. A small shield-shaped area separates them in the middle line, but otherwise they cover the entire width of the dorsum. Viewed from above they cover and conceal the entire head and curve downward in front of the prostomium and beneath the tentacles. All are very broad and flat at the base, especially the two inner ones on each side. In the case of three of them the outer one-third is less flattened and tapers to a blunt point. The anterior median of both sides, however, expands into a broad flat plate (fig. 56) somewhat lobed on the margins and terminated by a tapering process bent sharply on itself into a hook. This process is complete on one side only, having been broken off of the other. Probably all of the branchiæ are straight in life, but they are peculiarly stiff and rigid and their cuticular covering is thick and marked by fine transverse striæ, but otherwise smooth.

Thoracic parapodia consist of simple setigerous tubercles arising from the lateral muscular shelf and short auriculate tori with both dorsal and ventral angles produced somewhat freely. Abdominal parapodia (fig. 57) consist of prominently projecting hatchet-shaped ventral tori, each bearing a minute cirriform process, and sharply bent, clavate dorsal cirri which replace the setigerous tubercles of the thoracic region. Parapodia of successive somites are united by a beaded muscular ridge which passes along the side of the abdomen between the torus and cirrus. The worm consists of thirty-two segments, of which II bears the paleoli, III to XIX ordinary setæ, and VI to XXXII uncini.

The paleoli (fig. 58) are of a clear, bright glistening straw color and are arranged in a half tubular figure at the base, from which they diverge and spread distally in a fan-shaped fascicle. In each tuft the shortest one (at the inner end of the posterior limb) is about twice as stout as the longest. Distally they all taper regularly and rather rapidly to very long, slender, acute and stiff tips. Except for these tips they are striated conspicuously in both the longitudinal and transverse direction.

The ordinary setæ (fig. 59) are few in number and form rather small close tufts. They are apparently similar on all segments, being rather slender with tapering stems and acute, tapering tips, striated somewhat obliquely, and provided on one side with a wing of moderate width marked with distinct oblique striæ.

The uncini are numerous on the thoracic segments (135 on X). They have the form shown in figs. 60 and 60*a*, being roughly triangular, with large bases, a prominent superior ligament process, a projecting rounded lower angle and long tooth-bearing border with five or six strong acute teeth, the lowermost of which is peculiarly bevelled and covers a short inferior ligament process which nearly touches its lower surface. Abdominal uncini (fig. 61) are fewer in number (78 on XXV), much smaller, and formed quite differently. They have small bases and high outstanding tooth-bearing rami, and apparently lack the inferior ligament process.

Several thick-walled mud tubes in the same bottle were probably fashioned by this species.

Type locality, Queen Charlotte Sound, off Fort Rupert, Vancouver Island, B. C., Station 4,201, 138 to 145 fathoms, soft green mud, sand and broken shells.

Chone gracilis sp. nov. Plate XII, figs. 62–66.

A very pretty small species resembling *Chone duneri* Malmgren in proportions, but differing from that species in the short, and broadly winged, barbless tips of the branchiæ. From *C. teres* Bush this species is easily distinguished by its much longer branchiæ, small number of abdominal segments and the more elongated form of the abdominal uncini.

The type specimen has a total length of 32 mm., of which the branchiæ make 12 mm. and the thorax 5 mm., the diameter for nearly the entire length being about 2 mm.

The branchial bases are simple and very low, being completely concealed by the high collar. Ten pairs of delicate branchiæ extend equally to a length of more than one-half of the body. The stems are united by a very delicate membrane for three-fifths of their length and the remainder is broadly winged on both sides. The barbs are very delicate and are absent from an acute foliaceous tip about 2 mm. long. Eyes are totally wanting.

A collar of the form typical of the genus rises to a height of two and one-half times the length of the segment which follows it. The dorsal ends are refolded into the dorsal sinus and a slight notch occurs in a median ventral position; otherwise it is quite simple. The very small tentacles are shorter than the diameter of the body.

Nine segments, 8 of which are setigerous, form the thorax and 51 segments the abdomen. For nearly its entire length the body is cylindrical and of almost uniform diameter, but in the posterior fourth becomes somewhat broader and depressed before tapering rapidly to the caudal end. No distinct glandular ventral plates are developed, but all of the thoracic and the anterior three or four abdominal segments are completely encircled by a thick glandular layer. The thoracic and a few anterior abdominal segments are strongly biannulate, the middle abdominal are simple and half as long as wide, while the posterior are much shorter and more crowded. The fecal groove is distinct on the caudal fourth of the abdomen, and conspicuous on a few anterior abdominal segments, also where it passes obliquely around the right side of the first abdominal segment and on the dorsum of the thorax; elsewhere it is faint or absent. A few small ova are present in the coelom. Every portion of this worm is white, without a trace of pigment anywhere.

All setæ tufts are short but rather prominent; uncinigerous tori are also short and nearly uniform, diminishing only slowly and regularly from the first to the last. The setæ and uncini are all delicate and colorless. The collar fascicle consists of a small number of narrowly winged, acute, capillary setæ. Remaining thoracic somites have a larger number of partly capillary, partly spatulate setæ placed between two small lappet-like processes. The former consist of a dorsal row of acute tapering setæ with one moderately wide and one just perceptible wing and a small number of very small and inconspicuous crooked setæ (fig. 62) with rudimentary wings found below the spatulated setæ. The spatulate setæ (fig. 64) are arranged in two rows of about ten each; they have delicate, striated, usually symmetrical, obovate blades, and long very slender mucronate processes. The abdominal setæ (fig. 63) are also in one series, few in number, rather coarser than the thoracic and with the two wings narrow and about equally developed. Thoracic uncini contain a single series of erect crochets or hooked setæ (fig. 65) with striated stems and the slightly enlarged head thrown back, a large coarsely divided crest and stout, blunt beak. On somite V there are 13 in a torus, on somite VIII 11. The abdominal tori contain uncini of the form shown in fig. 66, with quadrate bases and heads of much the form of the thoracic crochets but strongly recurved on the base. They are more numerous than the thoracic crochets, somite XXI containing 17.

The tube is nearly transparent, little tortuous and almost free from sand.

The single specimen comes from Station 4,274, Alitak Bay, Kadiak Island, 35 to 41 fathoms, on a bottom of green mud and fine sand. A smaller specimen was taken at Station 4,253, Stephens Passage, 131 to 188 fathoms, rocks and broken shells.

EXPLANATION OF PLATES X, XI AND XII.

PLATE X.—*Notophyllum imbricatum*—figs. 1–3.
Fig. 1.—Dorsal view of head of type. × 13.
Fig. 2.—Outline of parapodium of middle region, seen from in front. × 13.
Fig. 3.—Distal end of a neuropodial seta of average length. × 600.
Eulalia quadrioculata—figs. 4–6.
Fig. 4.—Dorsal view of anterior end of type. × 24.
Fig. 5.—Posterior view of parapodium from somite XXV. × 32.
Fig. 6.—End of an average seta from somite X. × 600.
Eulalia longicornuta—figs. 7 and 8.
Fig. 7.—Posterior view of parapodium from somite XXV of type. × 32.
Fig. 8.—End of a seta from somite X. × 600. Some of the setæ have even longer appendages.
Pionosyllis magnifica—figs. 9–11.
Fig. 9.—Dorsal view of anterior end of type. × 32.
Fig. 10.—Anterior view of parapodium of somite XXV. × 32.
Fig. 11.—End of a seta from the middle of a fascicle on XXV. × 600.
Stauronereis annulatus—figs. 12 and 13.
Fig. 12.—Anterior end of cotype from above. × 82.
Fig. 13.—A parapodium from somite XXV, anterior view. × 82.
Brada pilosa—figs. 14–17.
Fig. 14.—A medium-sized extended papilla from the dorsum of the middle region, showing ring of adhering silt. × 113.
Fig. 15.—An extended papilla from the ventral surface. × 113.
Fig. 16.—A portion of the middle of a notopodial seta from XV. × 250.
Fig. 17.—Exposed portion of neuropodial seta from XV. × 98. *a*, portion of middle of the same. × 250.

PLATE XI.—*Stauronereis annulatus*—figs. 18–22.
Fig. 18.—Distal portion of jaw from below. × 130.
Fig. 19.—Forked seta from middle of notopodial fascicle of somite XXV; *a*, another of the same slightly rotated and foreshortened. × 800.
Fig. 20.—Portion of the middle of a capillary neuropodial seta from somite XXV. × 800.
Fig. 21.—A short-bladed compound neuropodial seta from the middle of the fascicle of XXV. × 800.
Fig. 22.—A long-bladed compound seta from the same somite. × 800.
Travisia pupa—fig. 23.
Fig. 23.—A small portion from near the distal end of a notopodial seta from the middle region. × 800.
Notomastus giganteus—figs. 24 and 25.
Fig. 24.—An entire crochet from the middle of the body. × 333.

Fig. 25.—Profile view, and *a* face view, of the end of one of the same. × 800.

Maldane similis—figs. 26–30.

Fig. 26.—Slightly winged seta from II. × 333.
Fig. 27.—Hispid capillary seta from somite XV. × 480.
Fig. 28.—End of crochet from somite III. × 480.
Fig. 29.—Entire crochet from X. × 110.
Fig. 30.—Distal end of the same. × 480.

Maldanella robusta—figs. 31 and 32.

Fig. 31.—Two entire crochets from somite XV. × 110.
Fig. 32.—The end of one of the same. × 480.

Clymenella tentaculata—figs. 33–35.

Fig. 33.—Middle crochet from somite II, entire. × 110.
Fig. 34.—End of the same. × 480.
Fig. 35.—End of a middle crochet from V. × 480.

Nicomache coronata—figs. 36–39.

Fig. 36.—Small portion of a fiber seta from somite X. × 800.
Fig. 37.—End of a crochet from somite V. × 480.
Fig. 38.—An entire crochet from somite XV. × 110.
Fig. 39.—End of the same. × 480.

PLATE XII.—*Lumbrichymene pacifica*—figs. 40–42.

Fig. 40.—End of a spine from II. × 98.
Fig. 41.—An entire crochet from somite X. × 83.
Fig. 42.—End of the same. × 440.

Nicomache coronata—figs. 43 and 44.

Fig. 43.—Small seta from somite X. × 360; *a*, small portion of the same. × 600.
Fig. 44.—Large seta from somite X. × 360.

Sabellaria cementarium—figs. 45–51.

Fig. 45.—Three spines forming a middle segment of the operculum, shown in profile in as nearly as possible their natural relations; *i* inner, *m* middle, and *o* outer spines. × 32.
Fig. 46.—A face view of the end of an outer spine. × 32.
Fig. 47.—Middle notopodial seta from somite III. × 32.
Fig. 48.—Portion of an ensheathed capillary notopodial seta from somite XX. × 440.
Fig. 49.—Bipinniform neuropodial seta from II. × 98.
Fig. 50.—A portion of the middle of the same. × 440.
Fig. 51.—Middle abdominal uncinus. × 440.

Samytha bioculata—figs. 52 and 53.

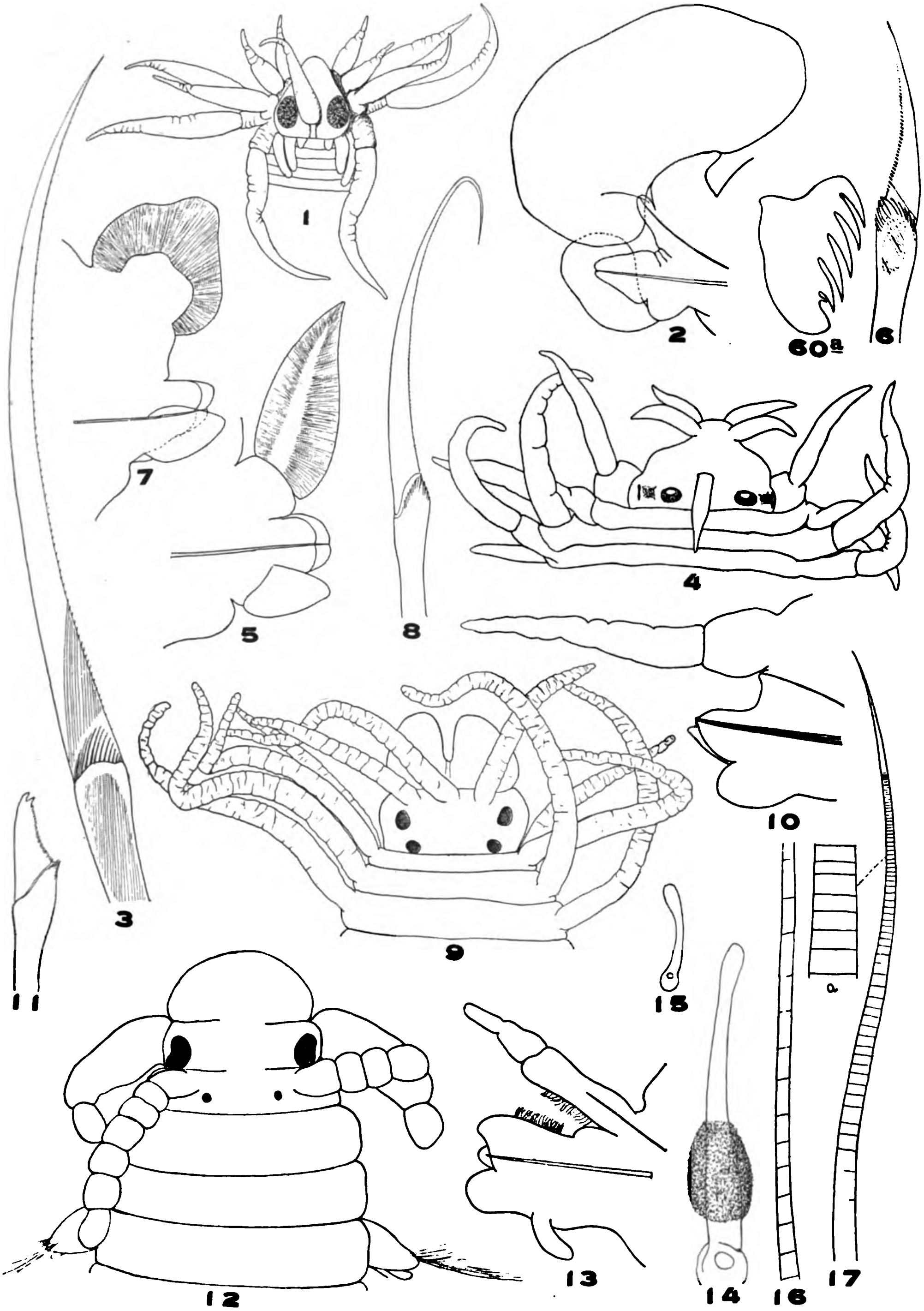
Fig. 52.—Uncinus from somite X of type. × 600; 52, the same from somite X of the cotype, somewhat foreshortened. × 600.
Fig. 53.—Uncinus from somite XXV. × 600.

Amphicteis scaphobranchiata—figs. 54-61.

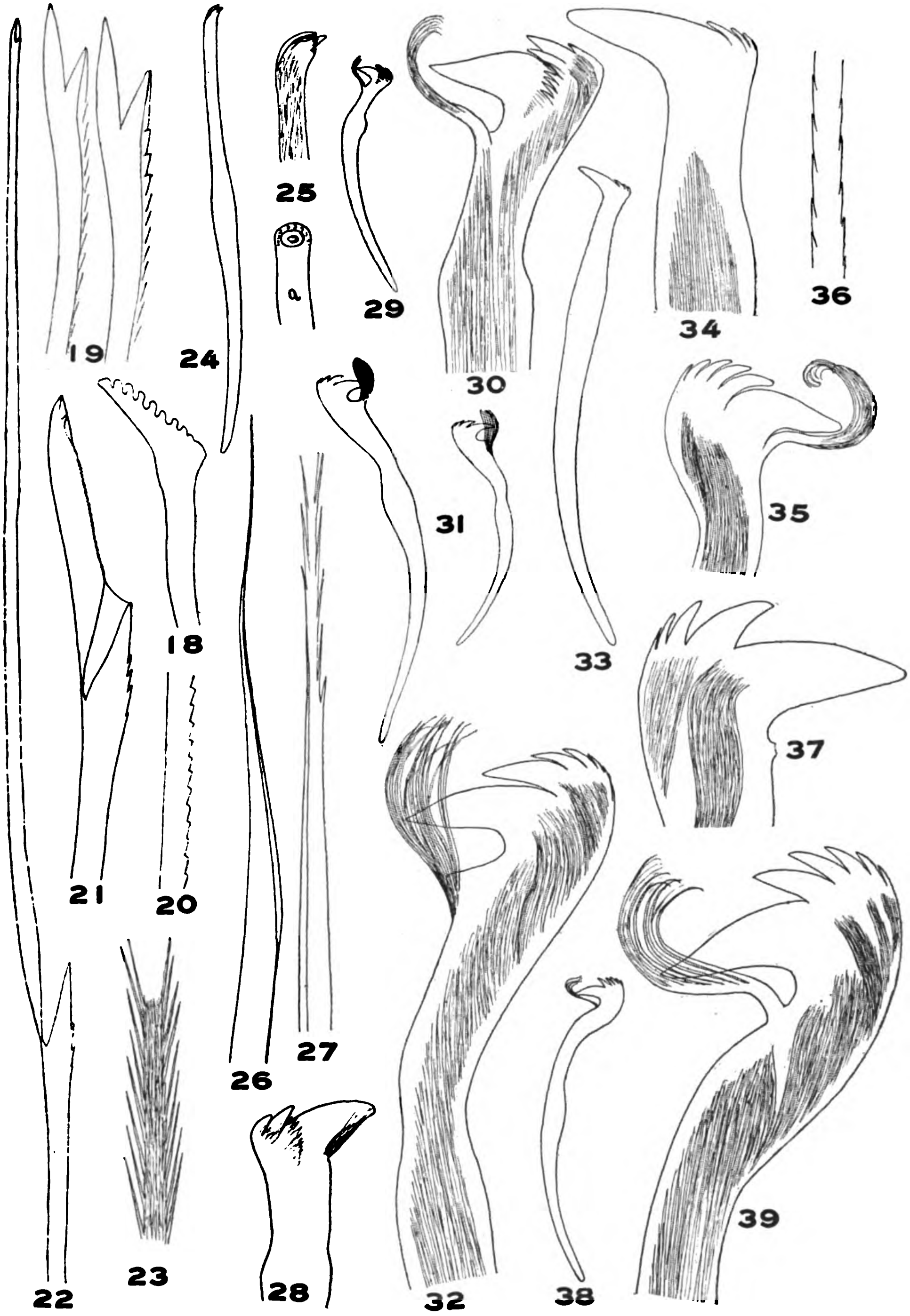
Fig. 54.—Dorsal aspect of anterior end of type, with branchiæ cut away and separated to show the prostomium. × 9.
Fig. 55.—Ventral aspect of the same with the branchiæ in place. × 9.
Fig. 56.—Distal portion of one of the anterior middle branchiæ. × 9.
Fig. 57.—One of the abdominal parapodia. × 24.
Fig. 58.—A paleolus from the middle of the fasciculus. × 83.
Fig. 59.—A seta from somite X. × 250.
Fig. 60.—Two forms of uncini from somite XII, the five-toothed one i somewhat foreshortened. × 600.
Fig. 61.—An uncinus from XXV. × 600.

Chone gracilis—figs. 62-66.

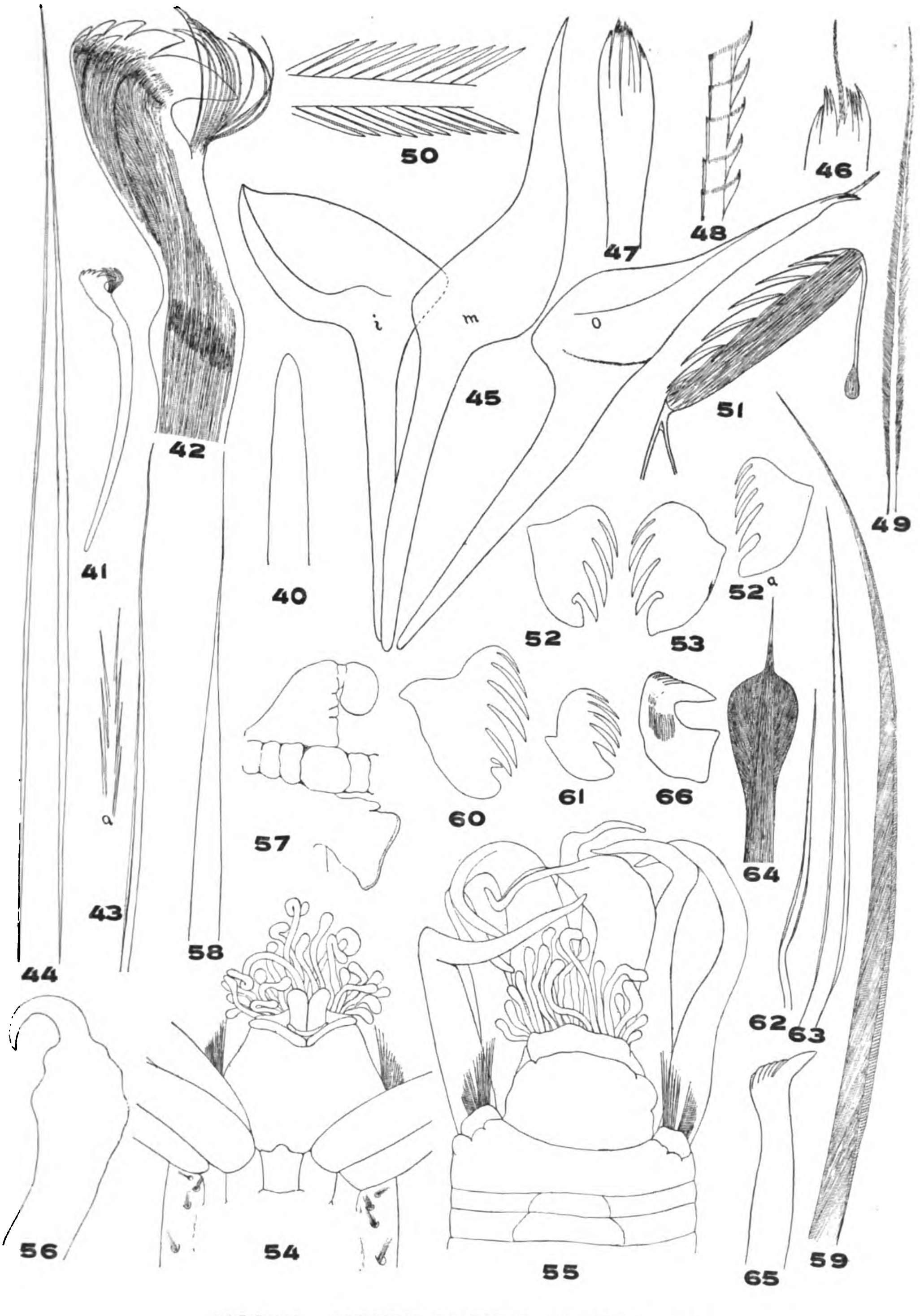
Fig. 62.—A small bent seta from somite V. × 360.
Fig. 63.—A winged seta from XXI. × 250.
Fig. 64.—A spatulate and mucronate seta from VI. × 360.
Fig. 65.—A crochet from VI. × 360.
Fig. 66.—An uncinus from XXI. × 360.



MOORE. NORTH PACIFIC POLYCHÆTA.



MOORE. NORTH PACIFIC POLYCHÆTA.



MOORE. NORTH PACIFIC POLYCHÆTA.

From the Proceedings of The Academy of Natural Sciences
of Philadelphia, July, 1906.

Issued September 28, 1906.

DESCRIPTIONS OF TWO NEW POLYCHÆTA FROM ALASKA.

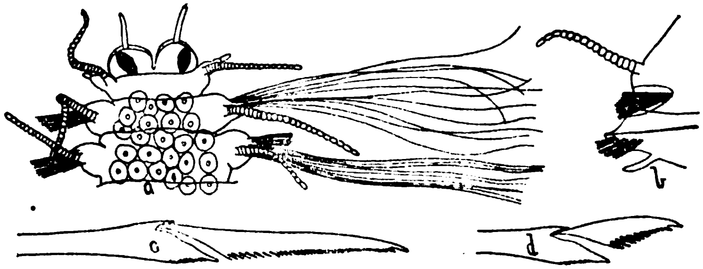
BY J. PERCY MOORE.

Among a number of Alaskan Polychæta in the collections of this Academy, the two of which the descriptions follow are new.

Syllis quaternaria sp. nov.

This sexual free-swimming form of the type for which Malmgren instituted his genus *Chætosyllis* is probably the epitokous form of a true Syllis, but it cannot be correlated with any atokous form hitherto described.

The length varies from 7 to 9 mm.; the width between the ends of the parapodia is .8 mm. and between the tips of the capillary setæ 4.5 mm., or one-half the length of the body.



Syllis quaternaria.—*a*, dorsal view of prostomium, peristomium and first two somites bearing capillary setæ, × 24; *b*, parapodium from IX with setæ cut off, × 32; *c*, a long and *d*, a short notopodial seta from X, × 600.

The prostomium (*a*) consists of a pair of prominent hemispherical lobes separated by a deep, narrow, median cleft, and each again divided by a shallow transverse groove. A pair of simple unjointed or obscurely wrinkled cylindrical tentacles arise from the latter grooves and incline decidedly toward the dorsum. They reach a length of 1¼ to 1⅓ times the prostomium. No other cephalic appendages exist, but there are two pairs of dense, black or deep brown, short spindle-shaped eyes, each provided with a small cuticular lens. The dorsal eyes are the more posterior and look vertically upwards; the large ventral pair look downwards, forwards and outwards. Sometimes 1 or 2 small, rod-like bodies of pigment occur with the eyes. The mouth is a minute opening situated at the very bottom of the vertical prostomial cleft near the ventral surface.

The peristomium (*a*) is but little modified and a segment just like the others, except that the parapodia lack the capillary setæ. The sides of the body are nearly parallel almost to the caudal end, where the last 12 to 15 of the total number of 40 to 43 segments taper to the pygidium. Each segment is about ½ as long as wide, or anteriorly rather more than this. The small triangular pygidium bears a pair of large, stout moniliform cirri of about 25 joints, and in the males an additional short, slender, and unjointed median ventral cirrus.

Excepting the first pair the parapodia (*b*) are biramous, and all but the first and the last few bear capillary setæ in the notopodium. The basal part is a rather large dome-shaped structure, from which the neuropodium projects rather prominently and ends in a short, slightly curved dorsal acicular process, while a postsetal lamina, stepped at the ends for the rows of setæ, forms its more ventral portion. Two or three straight aciculi support the neuropodium. From the middle of the ventral surface of the foot arises a short, slender, tapered and unjointed ventral cirrus. The notopodium is a very slight, conical tubercle which receives the end of a single aciculum strongly curved at the tip and caps a large yellowish setigerous sac, from which the capillary setæ spring. Well above and separated from this tubercle arises the dorsal cirrus, which is always prominent and strongly moniliform. With considerable regularity the dorsal cirri are alternately longer and shorter, the even numbers being about equal to ⅔ of the width of the body exclusive of the parapodia and consisting of 20 to 25 joints, whereas the odd numbers are about ⅔ as long as they and have only 14 to 16 joints.

As stated above, all but the peristomium and the last 5 or 6 segments bear capillary notopodial setæ which form dense and very conspicuous bundles. They are perfectly smooth and simple in structure, glass-like in appearance, tapered to excessively fine but apparently flattened ends, and are more or less curved and sometimes curled and twisted at the ends—the latter condition being probably the result of preservation.

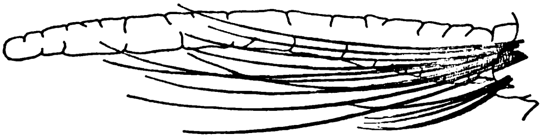
Neuropodial setæ are arranged in 5 or 6 horizontal sub-acicular rows. They are relatively short and stout and perfectly colorless and vitreous. The curved stems are deeply cupped, and toothed along the more prolonged side. The blades end in a single, rather stout, little hooked tooth, and the dorsal or shorter side is provided with a moderately strong fringe best developed on the shorter blades, which also exhibit an aggregation of some of the terminal hairs to simulate an accessory tooth. On the ventral setæ the blades seldom exceed a length of twice the diameter of the end of the shaft, while those of the 3 or 4 setæ in the dorsalmost row are narrower and 4 or 5 times as long as the distal end of the shaft.

Although all of the specimens examined are fully mature and have the body cavity filled with ova or sperm, the alimentary canal is complete and well developed, extending continuously from mouth to anus without exhibiting any of that occlusion or degeneration seen in many sexually mature syllids and other annelids.

The type and about a score of cotypes are No. 1,091 of the Academy's collections. Taken on the "surface in a lead 4 miles from shore by Mr. E. A. McIlhenny at Point Barrow, Alaska."

Ammotrypane brevis sp. nov.

The type of this species is a somewhat contracted specimen 15 mm. long, 1.5 mm. broad and 1.9 mm. high in the middle, being therefore rather robust and tapering to both ends. The general resemblance to *A. aulogaster* is close, but if the differences exhibited by the single specimen prove to be constant the two species are readily separated.



Ammotrypane brevis.—Parapodium and gill from somite X, × 56.

Instead of being laterally compressed the prostomium is blunt and flattened dorso-ventrally. From it a slight median ridge passes backward and gradually merges into the dorsal curvature of the back. Below this ridge on each side are the small sensory pits or nuchal organs. The mouth is a small trifid opening bounded by a pair of small anterior lobes and a single posterior lobe. Although the body seems to be quite complete and exhibits no indications of regeneration, there are only 29 setigerous somites not distinguishable from one another externally except through the presence of the parapodia. No intersegmental furrows whatever are discernible and the segments pass continuously into one another. Ventrally a pair of stout muscular bands, separated by a deep median groove, produce a somewhat sole-like surface. Above this the body is narrower and strongly arched and marked only by the small transverse muscle bundles, apparently 4 to each segment.

If perfect, as it appears to be, the pygidium presents striking characteristics. The large median spoon-shaped lobe of *A. aulogaster* is absent and represented only by a minute slender process. The lateral lobes are much larger, obliquely truncated above, and slightly indented at the end.

The parapodia arise from the dorso-lateral surface of the lateral muscle bands and are about equally well developed throughout. They form small but distinct bluntly rounded tubercles produced into a small, conical prominence and apparently unsupported by an aciculum. They bear only a small ventral cirrus and a prominent dorsal branchia, the small dorsal cirrus which has been figured for some species being absent. Branchiæ are absent from the first pair of parapodia, but are present on all of the others. They are largest on the middle segments, where they exceed a millimeter in length and the parapodium in diameter. They are slender and slightly tapering in form and provided along one side with a strongly developed longitudinal muscle, rendering them very contractile.

The setæ are all simple, colorless, tapering, capillary bristles, longitudinally fibrillated and soft in texture. They are arranged in two fascicles, a dorsal of 6 to 10 setæ which reach ¾ the length of the branchia, from the base of which they arise, and a ventral which arises from beneath the conical prolongation of the parapodium and contains 5 or 6 setæ less than ½ as long as the dorsal bristles.

Along the sides of the body between the parapodia, but nearest to the succeeding one, are small black or dark brown and very conspicuous eye spots, which are hemispherical in shape and indistinctly facetted on the surface.

The only specimen, a female filled with eggs, is No. 284 of the Academy's collection, taken by Dr. Benjamin Sharp at Icy Cape, Alaska, on August 17, 1895.

DESCRIPTIONS OF NEW SPECIES OF POLYCHÆTA FROM THE SOUTHEASTERN COAST OF MASSACHUSETTS.

BY J. PERCY MOORE.

Arabella spinifera sp. nov.

This species is known only from an incomplete worm consisting of the prostomium and fifty-one anterior segments and measuring 18 mm. long and 2 mm. in diameter, indicating a form less elongated than *Arabella opalina* Verrill.

Prostomium subconical, very slightly depressed, acute, more than twice as long as the basal width; sides straight; ventral longitudinal grooves close together and very faint. The noteworthy elongation of the prostomium may be abnormal, resulting from an injury at the base. Eyes two, rather large, conspicuous, situated on the dorsum close to the posterior border of the prostomium and separated by a space of less than one-third the basal width of the prostomium. Palps rudimentary, or at least not visible from the exterior, and enclosed by the margins of the lips.

Peristomium and somite II clearly separated by a deep furrow, both dorsally and ventrally; both simple apodous rings, the former slightly the longer. Posterior lip smooth. Fort-bearing somites all very short, about 5–8 times as wide as long, all clearly defined by distinct furrows, simple in structure, and decidedly more arched dorsally than ventrally, making a subterete body.

Parapodia begin on III. The first (Plate XIX, fig. 3) consists of a small but prominent notopodium, and a neurapodium which is divided into a short and stout, rounded presetal lobe supported by a single stout aciculum, and a similarly formed but slightly longer postsetal lobe, which is ventral and only slightly posterior to the former. Remaining parapodia (fig. 4) are prominent, and the postsetal or ventral lobe is elongated into a stout cirriform branchial organ containing a large vascular loop. For the length of the piece the parapodia undergo no change, except, first, a slight increase in size of the postsetal lobe and, second, a scarcely perceptible reduction of it in the last ten or twelve somites.

On the first two or three parapodia the acicula do not project beyond the surface, but the neuropodia of all others are supported by two deep yellow acicula, the ends of which are exposed as in certain species of *Drilonereis* and *Aracoda*. The dorsalmost (fig. 5, *a*) is much the stouter and the end is blunt; the more ventral (fig. 5, *b*), besides being more slender, is prolonged into an acute spinous process, which is frequently broken off. Further caudad the larger aciculum becomes still stouter and more distinctly curved. Two or three very fine acicula reach to the base of the notopodial tubercle (fig. 4).

On the first somite the setæ are all in a small, nearly vertical fascicle between the two lobes, but they quickly rotate to a more oblique position and divide into an oblique supra-acicular group and a horizontal subacicular group. The former usually contains two pairs of doubly curved, very acute, limbate setæ with strongly striated stems and finely denticulated margins (fig. 6); these are turned with profile dorsal and ventral. Subacicular setæ are two or three in a horizontal row, turned so that in preparations of the parapodia both blades come into view symmetrically; the blades are shorter and the tips more prolonged than on the supra-acicular setæ (fig. 7). Dorsal setæ yellow, ventral nearly colorless.

Mandibles (fig. 2) brown, with exposed tips white. The two halves merely touch without uniting and then diverge both distally and proximally; dentinal plate not clearly differentiated from the carrier and nearly equal to it in length; the former curved, divergent, ending in a blunt white tip, without marginal teeth; the latter relatively short, broad at distal, tapering to proximal end, divergent. Maxillæ (fig. 1) brown, four pairs, all but the first pair (forcep jaws) alternating in position and more or less asymmetrical. Carriers of forceps filiform, about twice the length of the series of jaws; the two halves coalesced near the end, thickened, then constricted and again enlarged at the distal end; the forceps roughly triangular, with three stout, hooked teeth along the medial margin of the basal half and the ends strongly hooked. The first accessory plate on the left side longer than the forceps plate, with a large hooked distal tooth and seven stout teeth gradually becoming smaller toward the proximal end. All of the remaining jaw plates differ little in size, but become successively somewhat smaller toward the anterior end; each is supported by two broad divergent wings and the dentinal ridge bears 4–6 conspicuous, slender, claw-like teeth, one or two of the anterior usually being enlarged.

Color of preserved specimen: anterior end and parapodia rich yellow, pale farther back; the cuticle only slightly iridescent.

The type and only known specimen (A. N. S. No. 2313) was found among a lot of *Ninoe nigripes* Verrill dredged on the muddy bottom of the middle of Buzzard's Bay, Mass., the exact spot being unknown.

Praxillella tricirrata sp. nov.

What appears to be an undescribed species of *Praxillella* is imperfectly represented by several fragments, one consisting of the head and nine metastomial segments, of which the head and five segments are in process of regeneration, a second of somites VI to XII inclusive, and a third of the pygidium and eight preanal segments, the first five of which are setigerous.

This is a larger species than *P. zonalis* or *P. elongata,* having a diameter of 3 mm. and an estimated length of about 150 mm. The only known anterior end, being in process of regeneration, presents an abnormal appearance, somewhat approximating that of a *Nicomache* or *Lumbriclymene.* The entire regenerating region, consisting of the prostomium and six somites, has a length of a trifle more than 3 mm. and barely exceeds the sixth segment.

Head very short, with the cephalic plate and limbate margin scarcely developed. There is a rather thick, short and broad palpode, and running back from it a narrower but, relatively to the width of the head, broad median ridge bounded on each side by the deep and conspicuous sensory clefts, which again are bounded laterally by the low folds from which the limbate margins will develop, the whole forming a narrow area scarcely exceeding one-third of the entire width of the head and sloping steeply downward anteriorly. Mouth a rather large crescentic opening bounded by the narrow, furrowed persitomial lip. Peristomium very short and uniannular.

Somites II to VI are about twice as long as wide, slightly compressed, distinctly biannular, decreasing slightly in diameter to the last, and with the furrows, except $\frac{\text{I}}{\text{II}}$ which is obscure, well defined. No distinct collars. The integuments of this region arc soft and delicate and a careful examination discloses no developed setæ, though the setigerous glands are visible on some segments.

The first normally developed segment (VI) is cylindrical, quite as long as the preceding part of the worm, and 2–3 times as thick; VII and VIII are quite similar; IX and X are united into one joint about four times as long as wide and without any dividing furrow; XI is slightly longer than VIII, and XII still longer. Except $\frac{\text{IX}}{\text{X}}$ all furrows in this region are deep and distinct; the segments somewhat depressed and distinctly flattened ventrally. On VI, VII and VIII the parapodia are situated one-third of the length of the segment from its anterior end, and all of the area anterior to them is occupied by a thick, whitish, glandular zone; a similar zone exists on IX, while on X a thick, rugous, ventral, glandular area of triangular form extends forward from and between the posterior tori to an apex halfway to the tori of IX, whence a narrow median ventral ridge continues it forward to the glandular zone of IX; XI and XII have no glandular areas, though the tori form prominent swellings near their posterior ends.

The posterior segments are much contracted and distorted, but it is evident that they consist normally of a slender, very contractile, and wrinkled anterior portion, and a short, enlarged, posterior parapodial portion. They decrease in length regularly to the pygidium, which is preceded by three achætous segments, of which the first closely resembles the last setigerous in size, and has posterior swellings representing the parapodia; the next is about one-half and the next one-fourth as long, widest posteriorly, where there are slight parapodial thickenings. The last two gradually widen into the base of the pygidium.

Pygidium (Plate XIX, fig. 8) with a short basal ring resembling the last achætous segment, and bearing a thick posterior anal disk, the margin of which is provided with a median ventral cirrus and a pair of lateral, long, slender cirri each having a length of ⅔ the diameter of the disk, together with a circle of very regular prominent teeth of which five are between the median and each of the lateral cirri, and fifteen between the two latter on the dorsum. Anus occupying the centre of a low, regular, finely furrowed cone, and closed by a thick, fleshy papilla, which arises from its ventral margin and bends dorsad. The usual raised neural line runs for the entire length and passes on to the ventral cirrus.

Setæ and uncini are undeveloped on the regenerating segments. They are sessile on VI–IX and borne on small papillæ and prominent tori on all the others. Anteriorly the setæ form small but very prominent, vertical, linear tufts, which become more compact posteriorly. They are similar on all segments. Anterior tufts contain 10–12 longer, stouter, slightly curved, narrowly limbate setæ (fig. 9), with long, capillary tips, and nearly twice as many slender, capillary, wingless setæ, partly smooth and partly bipinnate, with fine hairs (fig. 10). Farther back they become longer but fewer and in more compact bundles, and most of the wingless setæ are smooth.

The anterior thoracic crochets are of course unknown. On other segments there are 15–19, the larger numbers behind. They are pale yellow, strongly striated, with rather slender stem, slightly constricted to form a neck, but lacking a distinct shoulder; an expanded head with a short, stout beak; a low crest with about four large and two or three small, diminishing teeth flanked by small fibrous teeth; and a conspicuous guard composed of 5–6 stout flattened hairs united at the base and in contact with the lower surface of the beak to its tip, above which they arch and recurve in a bold circle (figs. 11 and 12).

Living color and tube unknown. Filled with eggs on Sept. 2. Taken on two occasions at Crab Ledge, east of Chatham, Mass., in 17–20 fathoms, on a stony and gravelly bottom, and not found elsewhere. (Type No. 1253, Academy of Natural Sciences of Phila.)

Cirratulus parvus sp. nov.

Form very slender, terete, nearly linear, but most slender and attenuated posteriorly. Length 20–30 (usually about 25) mm., diameter about .6 mm. Number of segments 60–75.

Prostomium and peristomium united to form a distinct head (fig. 13). Prostomium broad and flat, broader than long, its width nearly two-thirds widest part of body, shaped like the blade of a gravedigger's shovel; the upper surface with a pair of obliquely transverse sensory slits at the place of union with the peristomium; the ventral surface somewhat thickened and divided by a longitudinal fissure. Eyes, one pair, conspicuous, rounded or slightly elongated obliquely, widely separated on middle of head, close to the sensory slits.

Peristomium a simple, somewhat swollen ring, scarcely differentiated from the prostomium above, but elsewhere clearly defined and forming a straight and smooth lip behind the relatively large mouth. Next two segments (II and III) simple rings with a diameter equal to the peristomium and a combined length equalling the entire length of the head.

Setigerous and branchiate somites begin with IV, which is slightly enlarged. They are at first quite short, but rapidly lengthen without corresponding increase in diameter until the length nearly or quite equals the diameter. Posteriorly they again diminish in length and at the caudal end is a short region of not clearly defined segments. All somites simple and uniannular, and the intersegmental furrows smooth and clear cut. Although the body is nearly terete the ventral surface is somewhat flattened and the parapodia somewhat projecting. Pygidium bearing a minute pointed projection below a nearly circular, dorsal anus.

Parapodia begin on IV and continue to posterior end; they are small, ventro-lateral projections bearing separate notopodial and neuropodial tubercles.

Branchial cirri also begin on IV just above the parapodia, and a pair occurs on each segment for about twenty, after which they are borne on every second or third segment and finally more scatteringly nearly to the posterior end. Cirri of the middle and posterior region are borne at the posterior margin of the segments some distance above the parapodia. They are slender, filiform and round, often half as long as the body and with the base constricted so that they are easily detached.

Dorsal gills usually two pairs, sometimes a rudimentary third one on one or both sides, placed side by side on the dorsum of IV just above the parapodium and a little behind the branchial cirrus. They are slender at the base and swell to about twice the diameter of the branchial cirri, longer than the latter, longitudinally grooved for their entire length, and in contraction crenulate, wrinkled and more or less coiled in an open spiral.

Setæ and spines scarcely differ in form and structure from those of *Cirratulus grandis*. At the anterior end both notopodial and neuropodial tufts contain capillary setæ only—about 6–8 in each—of various lengths, the longest about two-thirds the body diameter. Spines appear in the neuropodium at about XII, and a few segments farther back each neuropodial tuft contains about four spines and four setæ, much shorter than on anterior segments (fig. 14). At the posterior end there are about two of each. In notopodial tufts the setæ become fewer and shorter from before backward, but usually spines appear only in those of the posterior one-third.

Color pale yellow or orange, usually with a greenish tinge, the integuments translucent, permitting the dark intestine to show through. Gills and cirri reddish from the contained blood.

Known only from the deeper waters of Vineyard and Nantucket Sounds, in from 10–19 fathoms, where it lives in colonies among the crevices of *Amarœcium pellucidum* and in passages of shells. Quite common in the latter at Crab Ledge, off Chatham. Nothing definite known of breeding habits, but specimens taken in late August contain small eggs. (Type No. 1657, Academy of Natural Sciences of Phila.)

This species is easily distinguished from young of *Cirratulus grandis* of the same size, which are frequently taken under stones at low water, by having but two achætous segments between the peristomium and first branchial segment. It bears a close superficial resemblance to *Cirrhatulus fragilis* Leidy, but differs decidedly in that the latter is described and figured as having bifid spines, only one apodous prebranchial segment, and the branchiæ beginning on the second setigerous segment. The species recorded under the name *Cirrhinereis fragilis* Quatrefages as having been dredged in Vineyard Sound, and considered by Verrill to be identical with *C. fragilis* Leidy, is probably the species here described.

Amphitrite attenuata sp. nov.

Form slender, clavate, slightly swollen in the anterior thoracic region, slender and tapering behind. Length 30 mm., diameter nearly 2 mm. at inflated part of thorax. Number of somites 80, IV–XXVIII setigerous.

Prostomium broad and depressed at the base, produced forward into a rather thick, simple lip, broadly rounded, entire and usually curved upward at the anterior margin, little folded about the mouth laterally and not meeting the lower lip; tentaculiferous ridge in contact with the lateral ends of the lip but separated from it by a slight notch, arched over the dorsum of the prostomium posteriorly and bearing in a continuous series about 30 coarse tentacles about one-half the length of the body. No eyes visible.

Mouth large, bounded below by a ventral lip which is divided by a transverse furrow into an inflated internal portion and a flat external portion, which is very broad and extends laterally beyond, but does not touch the upper lip. Peristomium a short, simple ring dorsally expanded ventrally to form the lower lip. Somite II as long as the prostomium and ventral lip combined, its anterior margin without a true collar or lateral wings, but slightly produced as a free rim. Thoracic region distinctly segmented, the furrow complete and well defined; dorsal region most inflated at X–XII, thence gradually tapered to the abdomen. Abdomen attenuated, nearly quadrate in section, the dorsum only rounded; the segments becoming very short but all distinctly biannulated, the posterior borders wider and somewhat projecting. Pygidium a simple, rugous ring.

Ventral plates 14 (II–XV) all, except the first, sharply defined but in contact with one another; the anterior ones rectangular, 2–4 times as broad as long, the last four elliptical and one and one-half times to twice as broad as long. Posterior to the ventral plates a ventral groove with a raised neural line continues to the posterior end.

Gills (fig. 15) three pairs, on II, III and IV, decreasing in size from before backwards, all small; when fully extended the first not more than one-half the thoracic diameter, each with a short trunk dividing near the base into 2–4 main branches, each of which then dichotomizes, usually three times and terminates in slender, tapering twigs.

Setigerous papillæ begin on IV just beneath the last gill, and are represented in a corresponding position on III by a minute achætous, knob-like papilla. Anteriorly they are prominent and at a high level; posteriorly they shift ventrad and become smaller. First torus on V, about one-half as long as the longest on XII, and one-sixth or one-seventh the body circumference. First five tori separated ventrally by about three times their length, those on XII by an interval about equal to their length and succeeding ones by a less distance. Posterior to the region of ventral plates they become more elevated and prominent, but, though assuming a lappet-form, they never become largely free, even on posterior abdominal segments.

The characteristic setæ arise from short vertical lines in tufts of about a dozen arranged in double series. They (fig. 16) have remarkably broad limbi, those of the posterior row being shorter than and half again as broad as the one figured; the very slender and much attenuated tip is very long, and the fringe nearly obsolete and confined to the outer end on the long setæ, but better developed, though exceedingly delicate, on the short ones.

Uncini occur in single series pointing forward on V–X, in double series alternating in position and facing each other on XI–XXVIII, then again in single series to the end; there are about thirty-five on V, sixty on X and XI, thirty-six on XVIII and twenty on L. They are nearly colorless, with short, strongly convex bases deeply notched in front, the beak stout, the crest of four transverse rows of 3–9 teeth and the guard short and broad (fig. 17).

Color pale reddish salmon, deepest anteriorly, tentacles flesh color.

This species has been taken only on the piles of the New York Yacht Club wharf in Vineyard Haven, where it occurs sparingly below low water among *Cynthia* and *Amarœcium*; lives in small mud tubes and breeds in early July.

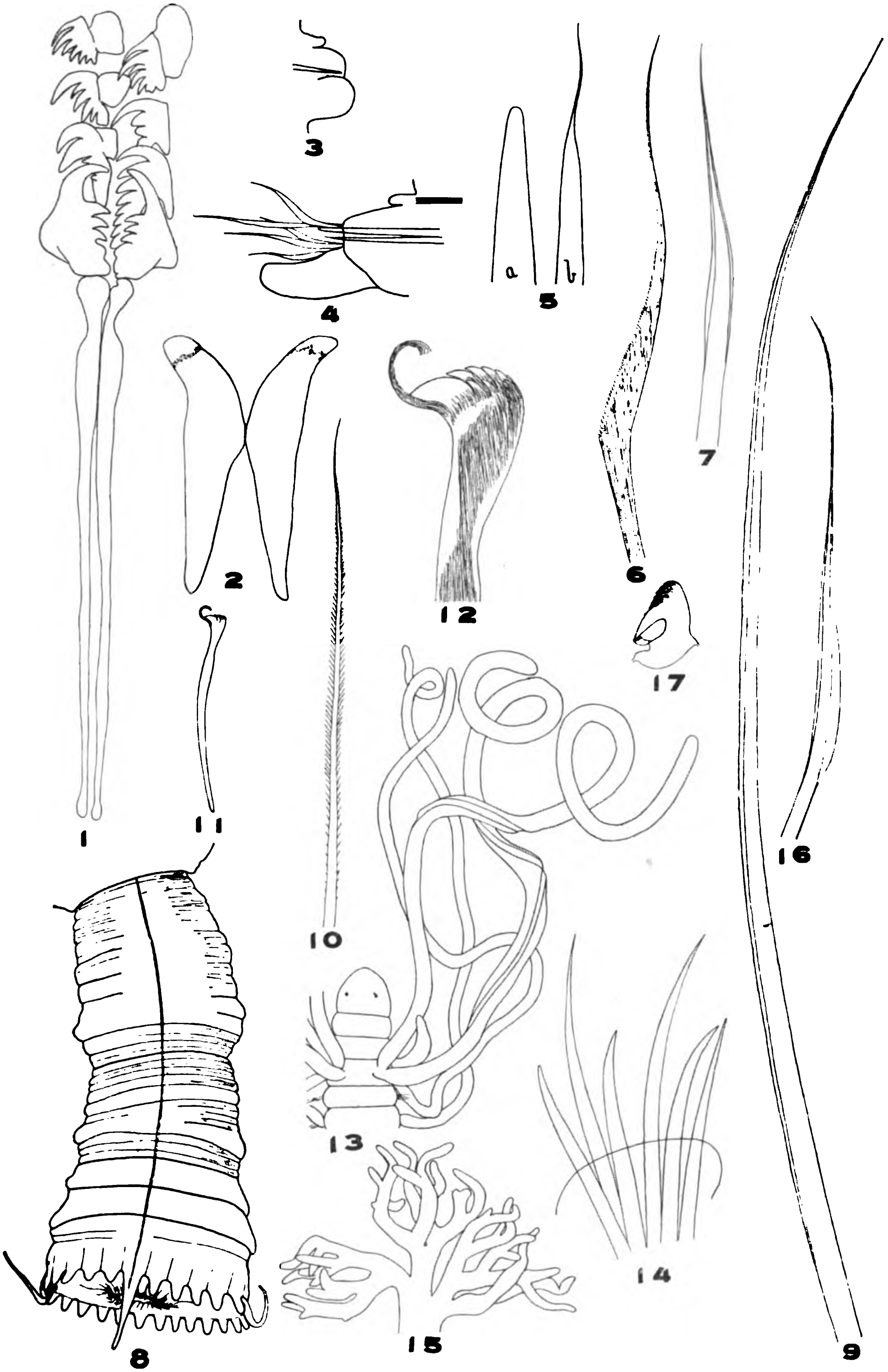
EXPLANATION OF PLATE XIX.

Arabella spinifera—figs. 1 to 7.
Fig. 1.—Ventral view of maxillæ, the accessory jaws slightly turned by pressure. × 56.
Fig. 2.—Dorsal view of mandibles. × 56.
Fig. 3.—Outline of parapodium III, posterior aspect. × 56.
Fig. 4.—Anterior view of parapodium X. × 56.
Fig. 5.—Distal ends of the two acicula. × 250.
Fig. 6.—Profile view of a moderately elongated seta from X. × 250.
Fig. 7.—Face view of a short subacicular seta from X. × 250.

Praxillella tricirrata—figs. 8–12.
Fig. 8.—Pygidium and caudal achætous segments. × 9
Fig. 9.—Slender capillary seta from XI. × 250.
Fig. 10.—Bipinnate capillary seta from XI. × 440.
Fig. 11.—An entire crochet from XI. × 82.
Fig. 12.—End of crochet. × 360.

Cirratulus parvus—figs. 13 and 14.
Fig. 13.—Anterior end showing the branchiæ and branchial cirri. × 24.
Fig. 14.—A tuft of notopodial cirri from the middle region. × 250.

Amphitrite attenuata—figs. 15 to 17
Fig. 15.—First gill of the left side. × 24.
Fig. 16.—A thoracic seta in ¾ view. × 360
Fig. 17.—A thoracic uncinus. × 360.



MOORE. NEW POLYCHÆTA.

DESCRIPTIONS OF NEW SPECIES OF SPIONIFORM ANNELIDS.

BY J. PERCY MOORE.

The following descriptions of four species of Spionidæ and one of Magelonidæ are based upon material mostly included in the collection of Polychæta belonging to this Academy, and which, with one exception, was secured by the writer in the region about Wood's Hole, Massachusetts.

Prionospio heterobranchia sp. nov.

This very interesting and distinct species is based upon a single imperfect specimen lacking the posterior end, and having a length for the first 65 segments of 12 mm., and a maximum width at somite VIII of less than ½ mm. Form very slender, widest and depressed in the branchial region, thence tapering very gently into the very slender and subterete posterior region.

Prostomium (Plate XV, fig. 1) elongated cuneiform, tapering behind to a slender point which overlaps somite II and reaches to its posterior margin; lateral margins with slight concavities opposite the eyes for the accommodation of the bases of the tentacular cirri; anterior margin entire and broadly rounded; a conspicuous median ridge begins just anterior to the eyes and, becoming more prominent, reaches to the posterior end of the prostomium, where it forms a slightly free and elevated process. Eyes one pair, very large and conspicuous, elongated, black, situated close together at the sides of the median ridge and between the bases of the tentacular cirri. No cephalic appendages. Peristomium closely united to prostomium, relatively small, somewhat pushed forward below as an oral papilla in the form of a truncated cone; at the sides and above embracing the prostomium laterally as slender, curved, lateral cephalic lobes much shorter than the prostomium and ending in rounded enlargements anterior to the attachment of the tentacular cirri, which have unfortunately been lost. Proboscis protruded in the form of a short inverted cone with a somewhat crenulated border extending slightly beyond the prostomium.

Somites II to VI wider and much depressed; VII to X of the same width but becoming deeper; after X the segments become gradually more slender and terete and taper gently caudad. Branchial segments very short, the others about ⅔ as long as wide and feebly marked.

Parapodia of branchiate segments II to V large and conspicuous (figs. 2 and 3); both notopodia and neuropodia provided with large, foliaceous, postsetal laminæ, usually overlapping or at least touching across the sinus; the former rising as a long dorsal process external to but quite free from the gill; the latter nearly circular, and in the case of the first one (II) apparently bearing a slender cirrus-like process on the ventral border. Behind V the parapodia are much smaller and soon become reduced to the form shown in fig. 4, in which the notopodial and neuropodial postsetal lamellæ have a similar form, but the former is twice the size of the latter. Posteriorly they become still smaller.

Branchiæ 5 pairs, on somites II to VI inclusive. All large, exceeding the diameter of the body even in contraction, erect, and arising from the dorsum close to the notopodia. The first pair (fig. 2) have a central stem containing a large vascular loop and ending in a slender, free filament. The basal ⅔ of the stem is crowded, chiefly on the posterior and medial surface, with numerous slender, finger-like processes, each with a small vascular loop connected with the two limbs of the stem loop; free surfaces ciliated. The last pair are similar, but the papillæ are longer toward the base and distributed on all sides of the proximal ⅔ of the stem (fig. 5). The 2d and 3d (fig. 3) are simply flattened and ligulate, marked across the posterior face by numerous very fine transverse ridges which correspond with delicate branches uniting the two sides of the vascular loop. The 4th is intermediate, resembling the last in form, but bearing a few filaments along its inner and posterior margin.

Anterior parapodia bear capillary setæ exclusively, arranged in shaggy, fan-shaped, vertical tufts in both rami, and curving outward and upward, the dorsal members of bundles on the branchial notopodia being especially long, and those of the first two exceeding the width of their respective somites. They are all simple, with very narrow wings or none, and very acute. Behind the branchial region the notopodials become gradually, and the neuropodials quickly, smaller and fewer. At about XL 1 or 2 crochets appear in the notopodium along with several very long, thread-like, flexible setæ. Little further change occurs within the length of the specimen. In the neuropodium crochets appear at XV; at XXV there are 6 or 7 crochets alternating with as many very small, straight, delicate setæ; at L there are about 10 crochets and a few still smaller setæ. Crochets (fig. 6) colorless, slender, terminated by 1 large and 3 or 4 small teeth, all enclosed in a spacious hood of two halves.

Body walls generally colorless and translucent, allowing the red blood in the anterior part and the olive-green intestine in the posterior part to show through; tentacular cirri with a red central vessel and gills red throughout.

The type and only specimen (Coll. A. N. S. P., No. 850) was dredged from the soft ooze at the bottom of the deepest part of the Eel Pond at Wood's Hole on August 4, 1902. Several subsequent searches have failed to reveal any others.

Prionospio tenuis Verrill, the only species definitely described from our shores, has four minute eyes and four pairs of gills, of which the first only is branched, the others being foliaceous. A larger specimen, probably representing a distinct species, is recorded as having all of the 4(?) pairs of branchiæ lanceolate and pectinate posteriorly, with slender papillæ. Webster and Benedict record an undetermined species at Provincetown, Massachusetts. The four or five European species are all easily distinguished by the characters of the prostomium, gills and crochets.

Numerous species of *Polydora* have been described from both sides of the Atlantic. Eight species occur in the region about Wood's Hole, the following two of which are new and rather closely related.

Polydora anoculata sp. nov.

Form very slender and elongated, anterior half depressed and of nearly uniform width; posterior half gently tapered, nearly terete. Length up to 20 mm., breadth at VI 35 mm. Number of segments about 98.

Prostomium (Plate XV, figs. 7, 8 and 9) about 3 or 4 times as long as broad, not prolonged caudally beyond somite II, but truncate or broadly rounded; anterior half abruptly bent downward, with a steep front; sides straight or slightly concave in anterior half; anterior end cleft medially, the halves diverging as a pair of rather prominent, short, rounded lobes, which may be drawn together or separated. Eyes totally absent and no nuchal ridge nor nuchal cirrus.

Peristomium (figs. 7, 8 and 9) bounding mouth by a small simple posterior lip; lateral cephalic lobes nearly meeting below, compressing the ventral portion of prostomium between them and extending as far forward as the base of anterior prostomial lobes. Tentacular cirri arising from posterior dorsal region of peristomium, in contact with sides of prostomium, rather stout, length three or four times width of body, reaching to XIII or XV, constricted at base, then widened, then tapered to blunt tip, channeled for entire length, and much wrinkled transversely.

Segments all well marked, uniannulate, those of the anterior ⅓ depressed, the remainder terete or subterete, strongly convex below, more or less flattened above; the first 4 very short, about five times as wide as long, nearly straight laterally; the next (VI) enlarged, nearly twice as long as the preceding, strongly convex laterally, and widest in front. The others gradually increase in length until the middle ones are ½ to ¾ as long as wide. Posteriorly they again become very short and close to the pygidium minute. Pygidium (figs. 10 and 11) bearing a small, scarcely expanded, somewhat funnelform caudal disk as wide as the small posterior segments and less than one-half the greatest width of the body, the margin even, with only a very faint ventral emargination and a deep dorsal cleft which passes into the slight fecal groove. Anus dorsal of the center, on a small papilla.

Parapodia of II with rami widely separated, the notopodium (fig. 7) immediately behind the base of the tentacular cirrus, and consisting of a minute setigerous tubercle and a short papilliform cirrus or postsetal lobe; the neuropodium at the level of the notopodia of succeeding somites, but otherwise of normal form. Succeeding parapodia are strictly lateral, with well separated rami; the notopodia at first with rather large foliaceous, postsetal lamellæ projecting dorsal, and after the gills appear gradually diminishing (fig. 12); neuropodia with longer lower postsetal lamellæ which practically disappear after somite VII. Branchiæ (fig. 12) appear on X on all specimens studied, the first very small, and continue to LIII at least. When best developed they are short, thick, digitiform and extend over the dorsum, meeting or nearly meeting in the middle line. They have the usual structure.

Somites II–V and VII bear capillary setæ only. Notopodials of II 5 or 6, very slender, delicate, and wingless; the others bear 4–6 longer, slender, slightly curved, and narrowly bilimbate dorsal setæ, and about as many shorter, coarser, often twice-bent, bilimbate ventral setæ. Neuropodia bear 12 or 14 setæ in 2 rows; they are similar to the notopodials, but, except on II, distinctly shorter and more strongly curved. Behind VII the notopodal setæ (figs. 13) are at first similar, but after the gills appear their number is reduced and they gradually become longer, more slender and straighter, those of posterior segments exceeding the diameter of the body. No notopodial crochets. Crochets appears in the neuropodia at VIII, which, like the next 3 or 4 segments, contains 2 or 3 accompanied by 1, or rarely 2, very delicate setæ at the ventral end of the series; for the remainder of the length there are 3 or 4 crochets and no setæ. Crochets (fig. 17) are delicate, colorless, strongly bent near the end and provided with a stout tooth arising at a wide angle (about 110 degrees), and above it an accessory tooth; both enclosed in a delicate hood. On VI from 4 to 6 sessile spines are arranged in a slightly oblique series, diminishing in size from above downward. They (figs. 16 and 23) are pale yellow, rather slender, the blunt tips rather strongly hooked, a prominent subterminal spur on one side, and a smaller one often on the other. Guard setæ (fig. 15) form a row anterior to and alternating with the spines; when unworn they are broadly bilimbate at the end with a delicate and elongated mucron. Overlapping the anterior-most spine from the dorsal side are 2 or 3 much larger bilimbate setæ (fig. 14), abruptly bent and either truncate or mucronate at the tip. A ventral posterior tuft beneath the last spine contains 4 or 5 much more delicate, nearly straight setæ, closely resembling the guard setæ.

Color of intestine usually pale yellow; body walls translucent, without pigment; blood red, coloring the gills and other parts.

Known from the region about Wood's Hole only. Uncommon. Usually taken in association with colonies of *Amarœcium pellucidum* in 7–17 fathoms in Vineyard Sound. Less often on piles among *Cynthia* in Little Harbor and with *Polydora colonia* at Vineyard Haven.

Type Coll. Acad. Nat. Sci. Phila., No. 2,338.

A specimen taken on July 14th contained nearly mature eggs.

Polydora colonia sp. nov.

Form elongated and very slender, resembling *P. anoculata* and of about the same size or rather smaller. Number of somites unknown. Length of head and 38 somites 6 mm., width at VI 3 mm. Depressed anteriorly, subterete farther back.

Prostomium (Plate XV, fig. 18) elongated; anterior half with straight sides and of nearly uniform width, posterior half tapering into a nuchal ridge which ends in a blunt point at the posterior margin of IV; anterior margin broadly rounded, entire or slightly emarginate. Eyes usually totally absent, but occasionally a small collection, or two, of pigment, as in the specimen figured.

Peristomium united with prostomium and somite II, forming a simple posterior lip and a pair of prominent lateral cephalic lobes much shorter than prostomium. Tentacular cirri attached to dorsum of lateral cephalic lobes, not constricting prostomium. The basal half has the usual structure and proportions, but the distal half, probably owing to maceration, is very slender and elongated, the total length being about 7 times the width of VI (fig. 18). The next four segments are very short and much depressed; VI is nearly as long as the three preceding segments, strongly convex and furrowed laterally, widest anteriorly. Succeeding segments rapidly increase in length until by XIII they are ⅔ as long as wide, becoming also subterete, strongly convex below and somewhat flattened above. Pygidium unknown.

Except the first (on II) the parapodia are larger than usual. That of II consists of a minute notopodial setigerous tubercle and papilliform postsetal lobe immediately behind the base of the tentacular cirrus, and a small neuropodium situated at the level of the notopodia of succeeding segments. Succeeding parapodia have prominent foliaceous postsetal lamellæ on both rami, but with the appearance of crochets on VIII the neuropodium becomes much reduced.

Gills begin on VIII or sometimes IX and continue to XXVIII, at least, arising from the dorsum in contact with the base of the notopodium; form subcylindrical, rather short, apparently never long enough to meet across the back, but their bases connected or nearly connected by a low, ciliated, delicate, transverse fold.

Setæ of II few and very small, the notopodials only 3 or 4, very slender and delicate, the neuropodials 6 or 8, short, slightly curved and narrowly bilimbate. Succeeding notopodial setæ rather conspicuous and numerous for the genus, anteriorly about 18–20, arranged in 2 rows, the dorsalmost (fig. 19*a*) longest and rising prominently over the back, narrowly bilimbate, very slender and little curved, the ventralmost (fig. 19*b*) much shorter, often doubly curved, with much broader wings and acute awn-like tips. Toward the middle of the body the number decreases and all the setæ become more slender and elongated. Anterior neuropodials also in two vertical rows of usually 6 or 7 each, all smaller and more strongly curved than the notopodials. Crochets appear abruptly in the neuropodium of VIII and never, so far as observed, are accompanied by capillary setæ. Anteriorly there are 3, farther back 5 or rarely even 6. They are colorless, slender, with a long acute beak and slender erect accessory tooth and are enclosed distally in a delicate hood, composed of a pair of well separated guards (fig. 22). On somite VI there are almost invariably three (in only one case out of many four) pale yellow spines in a strictly vertical row (figs. 20, 21). They are straight, slightly enlarged at the end where they bear a principal and an accessory blunt, straight, conical tooth, below which, on the posterior face, is a half-round sheath ending in a free border on the side of the small tooth and forming a flange encircling the base of the large tooth on the opposite side (figs. 20*a* and 21). On the anterior side of these spines and alternating with them are a corresponding number of broadly bilimbate setæ, and a group of 3 or 4 similar and perfectly straight setæ lies just dorsad of the spines. Ventral and slightly anterior to the lowermost is a second group of similar but much smaller setæ (fig. 20).

Lives abundantly in colonies formed of soft mucoid tubes covered with fine silt in the interstices of tunicates and sponges on wharf piles in the harbor of Vineyard Haven, Massachusetts. Individual tubes are less than 1 mm. in diameter and from 20–30 mm. long. Hundreds of them are inextricably intertwined and cemented together with mucous, forming masses 1 or 2 inches in diameter. Specimens taken at the end of July contain large eggs which begin at somite XVI or XVII.

The type is No. 2324 (Coll. Acad. Nat. Sci. Phila.).

Unfortunately all of the numerous specimens were preserved in the tubes and are more or less macerated and the posterior ends lost. The setæ, however, and especially the spines and associated setæ on VI, are perfectly characteristic and serve to distinguish the species completely from any European or American species.

Magelona rosea sp. nov.

Form very slender, body slightly depressed anteriorly, tapering very gradually and subquadrate posteriorly. Length 40 mm., greatest width 1 mm. Number of segments about 95.

Prostomium and peristomium completely coalesced to form a head (Plate XVI, fig. 24) of a broad ovoid form, about ¼ long erthan broad but capable of much greater elongation during life. A pair of dorsal cephalic ridges, in contact medially, extend for nearly the entire length of the head, tapering to acute points anteriorly, and near the end suddenly diverging, leaving a thin triangular area between. Anterior and lateral portions of prostomium formed by a thin but rigid shovel-like expansion marked on the dorsal surface by a number of delicate raised sub-parallel lines. No prostomial appendages and no eyes.

Peristomium dorsally consisting of the posterior portion of the cephalic ridges and a pair of prominent lateral lobes, again subdivided by a groove extending from the posterior margin; ventrally a simple ring bounding the mouth behind. Tentacular cirri (fig. 24) arising from the sides of the peristomium above the mouth and beneath the margins of the prostomial plate. They are 8 mm. long, reaching somite XX, delicate, and tapering, the lateral surface covered with very fine and close transverse ridges, the medial with somewhat coarser but still very small papillæ which increase in length to the distal half where they are as long as the diameter of the cirrus. Proboscis soft, bulbous, protruded to half the length of the head and marked with numerous fine longitudinal parallel ridges.

Metastomium slender and of nearly equal diameter throughout, only the anterior 8 segments and the tapered caudal end being distinctly narrower; margins of body even and regular, the parapodia being small and inconspicuous and arising from small, slightly depressed rings; segments more or less distinctly divided into dorsal, ventral and lateral fields by longitudinal furrows above and below the parapodia, the latter forming interpodal wrinkled glandular areas, and the ventral field divided by a median neural groove, which becomes obscure posteriorly. Anterior region, consisting of peristomium and nine podous segments, separated from the posterior region by a deep annular groove in which the ninth pair of parapodia lie (fig. 24); its segments somewhat narrower than those of the posterior region and without distinct intersegmental furrows, the distance between successive parapodia about ¾ the width. Ventral median field occupied by a series of slightly overlapping, segmental ventral plates, which become smaller from in front caudad and are divided into halves by the neural groove; lateral fields distinctly bounded and much furrowed transversely. Distinct glandular bands or half-rings extend across the dorsum between the parapodia, and several small and irregular transverse furrows mark the dorsum and sides of each segment, dividing it imperfectly into about 3 incomplete rings. Posterior region slightly wider than the anterior and consisting of 84 or 85 segments; first segment very short, but the others increasing in length until they are about half as long as wide; ventral field distinctly wider than dorsal, without ventral plates and divided by a deep ventral groove. Throughout most of this region the parapodia lie in the deeper furrows, and a shallower furrow is only obscurely indicated a short distance caudad to each pair. Farther back it becomes clear that the segments are biannulate and that the parapodia lie between the larger anterior and smaller posterior annulus.

Pygidium very small, oblique, with the anus dorsal and covered by a broad flat papilla; below and behind the anus a pair of small, slender cirri, often replaced by a pair of minute papillæ.

Parapodia all biramous and more or less foliaceous, the first 9 pairs with capillary setæ only, the others with crochets only in both rami. The first 8 pairs consist of winglike, foliaceous, postsetal laminæ, the notopodial being somewhat broader and strongly curved ventrad to meet the neuropodial, which is longer and projects more directly laterad; both somewhat contracted at the base to form a stem, which bears the spreading, fan-shaped tufts of setæ, the neuropodial being somewhat the larger. The ninth parapodium (fig. 26) is similar but rather larger and has a more extensive series of setæ. In the posterior region the neuropodia and notopodia consist of vertical ridges, each bearing a series of 18–20 crochets and ending in minute notopodial and neuropodial cirri, while from their contiguous ends arise foliaceous expansions borne on short stems (figs. 27–29). These expansions increase somewhat in size to the middle of the body and bending toward each other overlap broadly, but farther back they become much reduced in size and the cirri disappear, simultaneously with an increase in the number of crochets.

Setæ of both rami of the first nine pairs of parapodia (II–X) all capillary, colorless, moderately slender, not greatly elongated, with curved and tapered stems very delicately bordered by margins which are broad on the concave and narrow on the convex side; arranged in one regularly spaced series of from 15 to 30 and spreading in a broad fan-shaped figure. Except that they are slightly longer, the notopodials differ in no noticeable respect from the neuropodials, and except that they are rather longer, more numerous and in part arranged in double series, those of the tenth somite are exactly like the preceding ones. Posterior to somite X both rami bear hooded crochets only, in simple series facing each other; anteriorly each series contains 15 to 20, posteriorly as many as 30. They (fig. 30) are little exposed, colorless, slightly tapered distally, where they terminate in an abruptly hooked blunt beak, surmounted by a smaller tooth and enclosed in a pair of wide guards.

Color translucent white, the intestine varying from buff to greenish-brown, pharynx salmon pink, both showing through body walls; blood madder pink, especially conspicuous in the tentacular cirri which contain a large vascular loop.

Specimens taken during the latter part of August contain nearly ripe eggs in the middle segments of the body.

Type No. 1677, Coll. Academy of Natural Sciences of Philadelphia.

Lives in sand at and below low water, forming soft sand tubes under stones, etc. As the worm crawls the pharynx is everted as a tumid fold or bulb reaching half the length of the head, the ventral median part being most prominent and marked by delicate longitudinal furrows.

This species has been found only on a sandy beach, chiefly below low water, in a little shallow bay on the Buzzard's Bay side of Wood's Hole. Prof. E. A. Andrews found it at the same place about ten years before it came to my notice, and has described a specimen under the name of *M. papillicornis* Müller. The remarkable larvæ have been described by Fewkes (as *Prionospio tenuis*) from Newport, and by Andrews from Beaufort, N. C., and Wood's Hole.

Magelona papillicornis was originally described by Fritz Müller in 1858 from specimens taken off the coast of Brazil. Since then it has been repeatedly recorded from various parts of Europe, and because of its remarkable characters has been much studied. As mentioned above, Andrews was the first to recognize *Magelona* on the coast of North America. More recently Johnson has described a very distinct species from Puget Sound.

Hitherto the North Atlantic species has always been considered identical with the Brazilian species, the very noteworthy difference in the color of the blood of the two having been curiously overlooked. Müller describes his species as having pale violet blood, whereas all specimens from the North Atlantic have madder pink blood. Müller's description is very brief, and the other characters mentioned are generic only. It is quite probable that when the Brazilian species is better known other characters than the color of the blood will be found to distinguish it from ours. Comparison of Wood's Hole specimens with the excellent figures of parapodia given by McIntosh and Mesnil make it clear that the European species again is quite distinct from ours and will require to be named. The most obvious difference is that the setæ of the tenth somite of the former are provided with a bulbous terminal enlargement and small process, while those of *M. rosea* are unmodified.

Spionides japonicus sp. nov.

Known from the anterior end only. Size large, probably not less than 4 or 5 inches long, moderately slender. The type, consisting of the head and 44 setigerous somites, is 25 mm. long and 2.5 mm. wide. A fragment from the postbranchial region indicates a still larger worm, having a width of nearly 4 mm.

Prostomium (Plate XVI, fig. 31) flattened, subtriangular, nearly as broad as long, the base anterior; anterior margin very broad, divided into a low, wide, median lobe and a pair of narrow, bluntly rounded lateral lobes directed toward the sides; lateral margins concave, meeting in the blunt, rounded apex which is slightly elevated and bears a slender, suberect nuchal cirrus, behind which a low, narrow ridge continues along the dorsum. Eyes 1 pair, large, conspicuous, reddish-brown, crescentic, situated at a slight widening behind the middle of the prostomium; just anterior to each of them is a curved series of small reddish-brown pigment specks.

Peristomium forming lower lips and lateral cephalic lobes; the latter small, rounded laterally and becoming very narrow posteriorly, where they disappear beneath the prostomium just behind the level of the eyes. Tentacular cirri missing, their faint scars of attachment on the dorsum of the lateral lobes just anterior to the level of the eyes. Body segments short and rather indistinctly defined; much concealed by the foliaceous parts of the parapodia; body rather strongly depressed, gently convex above and below.

Parapodia very conspicuous, so large that they completely envelop the body and give somewhat the appearance of a series of thin disks strong upon an excentric band. They are very broadly attached and the two rami nearly continuous, with extended setigerous tubercles, low presetal lobes, and very large and foliaceous postsetal lamellæ. The first (fig. 31) is relatively small and lies by the side of the prostomium, the posterior part of which is crossed by the notopodial setæ. On the next few both postsetal lamellæ increase rapidly in size, the notopodial soon predominating over the neuropodial and extending dorsally and ventrally. In this region the neuropodial lamella (fig. 32) is formed much like the quadrant of a circular disk, and the notopodial lamella like a narrow palette, broadly rounded above and with an auricular process below which is absent or little developed in the most anterior parapodia. The notopodial lamellæ meet or overlap medially and touch the neuropodial lamellæ below. After somite XXX the notopodium gradually becomes smaller and withdraws from the dorsum. By XXXVIII it is strictly lateral and its postsetal lamellæ is no larger than that of the neuropodium, and leaves the dorsum entirely exposed. Simultaneously with this change a delicate transverse integumental fold appears on the dorsum, connecting the bases of the notopodial lamellæ of the two sides. At first quite insignificant, these increase in height as the branchiæ diminish and by XLII their lateral parts are nearly half as high as the lamellæ which they connect, but the medial part is much lower.

The first 3 pairs of parapodia are free, but all others are united continuously along each side by a thin lateral fold of integument, which is attached to the sides of the body ventrally and whose dorsal border is free between the parapodia but attached to each neuropodium for the full height of its base and for a short distance above it. Thus is formed a series of deep interpodal pockets widely open above.

Branchiæ arise from the dorsum just above the base of the notopodial lamella from III to XLII inclusive, largely concealed behind the medial ends of the lamellæ. The first 2 or 3 are rather small, but they soon increase in size and rise erect far above the notopodial lamellæ. The last 10 gradually diminish before they finally disappear. Typical branchiæ (fig. 32) are more or less flattened, ligulate, tapered and unbranched. They contain large axial bloodvessels, from which pinnate loops branch on either side, corresponding with slight surface plications.

Capillary setæ alone are found on both rami of parapodia of the branchial region. They form broad spreading fan-shaped tufts and are very numerous (40 or more notopodials and even more neuropodials) and arranged in two vertical rows, a long seta of the posterior being always paired with a shorter one of the anterior row; much the longest and most slender in dorsal part of notopodium, stouter in ventral part of both rami. All setæ are pale yellow with tapering, longitudinally striated stems finely granulated on the surface, with more or less prolonged and delicate tips and the more or less well developed wings or margins directed toward the middle part of the foot on both rami (fig. 33). Wings best developed on dorsal neuropodial and ventral notopodial setæ, the marginal setæ of both bundles nearly lacking them. Notopodial setæ of II very small and delicate, those of the two sides crossing over the prostomium. As the foliaceous structures and gills become smaller the setæ also become smaller and fewer. Crochets appear in the ventral portion of the series on neuropodia of the postbranchial region. There are 2 on XLI and 6 on XLIII. They (fig. 24) are nearly colorless, rather slender, delicately striated longitudinally, the tips gracefully hooked, with a stout beak surmounted by an accessory process, both enclosed in a delicate hood conforming to their contour.

The type is No.1, 055, Coll. Acad. Nat. Sci. Phila.; found with *Aricia fimbriata,* taken at Albatross Station, No. 5,771, off Honshu, Japan, on June 5, 1900; 61 fathoms; bottoms of green sandy mud.

From the type of the genus, *S. cirratus* Webster and Benedict, known only from Eastport, Maine, this species differs in having numerous pairs of gills beginning on III, instead of 13 pairs only beginning on IV, in having 1 pair of large eyes instead of 2 pairs of small ones, and in the much larger size.

EXPLANATION OF PLATES XV, XVI.

PLATE XV.—*Prionospio heterobranchia*—figs. 1 to 6.

Fig. 1.—Dorsal aspect of head and somite II, showing scars of attachment for tentacular cirri and gills. × 40.

Fig. 2.—Anterior aspect of parapodium II with setæ and detached gill of other side. × 98.

Fig. 3.—Similar view of parapodium and gill of IV, without setæ, × 98. Only a few of the transverse bloodvessels and external ridges are shown.

Fig. 4.—Anterior aspect of parapodium X. × 98.

Fig. 5.—Posterior aspect of detached gill of VI. × 98.

Fig. 6.—A crochet from neuropodium of L. × 600.

Polydora anoculata—figs. 7 to 17.

Fig. 7.—Dorsal aspect of head and first 6 setigerous somites, one tentacular cirrus in place. × 56.

Fig. 8.—Dorsal aspect of head and 2 setigerous somites of another specimen. × 56.

Fig. 9.—Ventral aspect of same. × 56.

Fig. 10.—Dorsal aspect of pygidium and posterior somites. × 56.

Fig. 11.—Ventral aspect of pygidium. × 56.

Fig. 12.—Parapodium XV. × 83.

Fig. 13.—Dorsal (*a*) and ventral (*b*) notopodial setæ from XV. × 440.

Fig. 14.—Strongly bent seta from dorsal tuft of VI. × 440.

Fig. 15.—Guard seta from VI. × 440.

Fig. 16.—Three spines from VI, *a* and *b* from young, *c* from adult (type) specimen. × 440.

Fig. 17.—Profile and face views of neuropodial crochet from XV. × 600.

Polydora colonia—figs. 18 to 23.

Fig. 18.—Dorsal aspect of head and first 6 setigerous somites, one tentacular cirrus in place. × 56.

Fig. 19.—Dorsal (*a*) and ventral (*b*) notopodial setæ from XV. × 440.

Fig. 20.—Anterior and ventral view, showing all of the spines and setæ of one side of VI under slight pressure, *a* the reverse or postero-dorsal aspect of the end of one of the spines. × 440.

Fig. 21.—Profile of one of the spines from somite VI of a larger specimen. × 440.

Fig. 22.—Profile and face views of a crochet from XV. × 600.

Fig. 23.—Variations in the form of the spines on somite VI of *Polydora anoculata*. × 440.

PLATE XVI.—*Magelona rosea*—figs. 24 to 30.

Fig. 24.—Dorsal aspect of head and anterior region, showing the right tentacular cirrus in outline and the left complete. × 9.

Fig. 25.—Ventral aspect of anterior end, showing bases of tentacular cirri, partly everted proboscis and ventral plates. × 24.

Figs. 26–29.—Outlines of parapodia X from the anterior side, all except fig. 27 with setæ or crochets. × 56.

Fig. 30.—Two crochets from somite L, striations omitted from one. × 440.

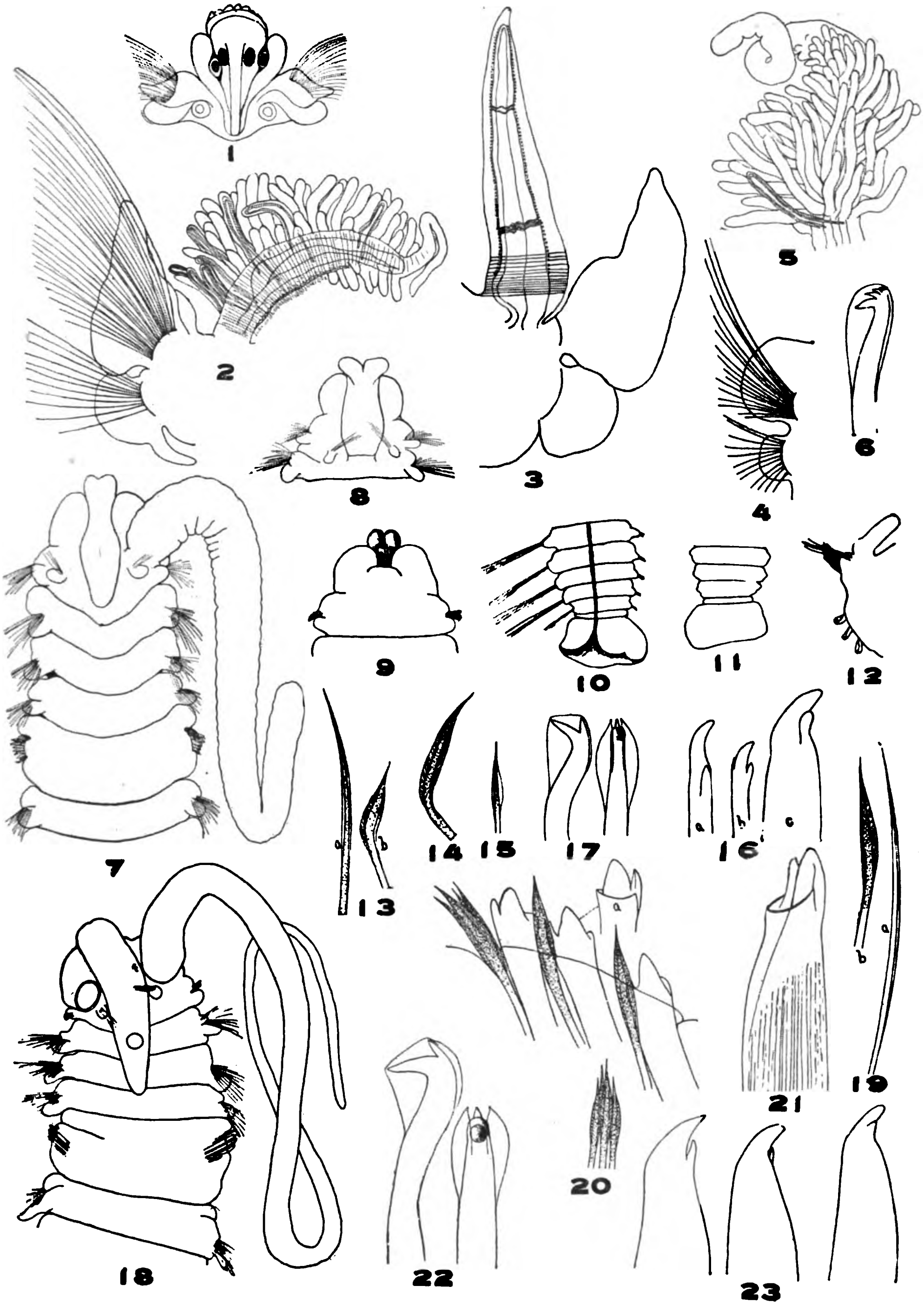
Spionides japonicus—figs. 31 to 34.

Fig. 31.—Dorsal aspect of head, lacking tentacular cirri, and first two setigerous somites with setæ. × 24.

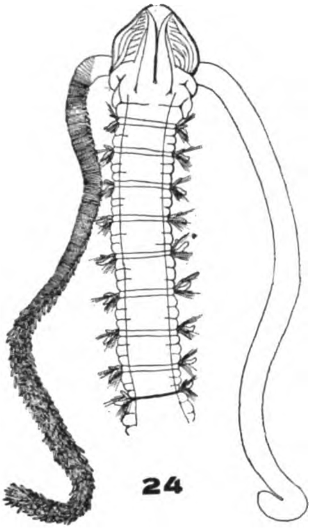
Fig. 32.—Anterior aspect of parapodium XV, with setæ, the concealed portion of the gill shown in dotted outline. × 24.

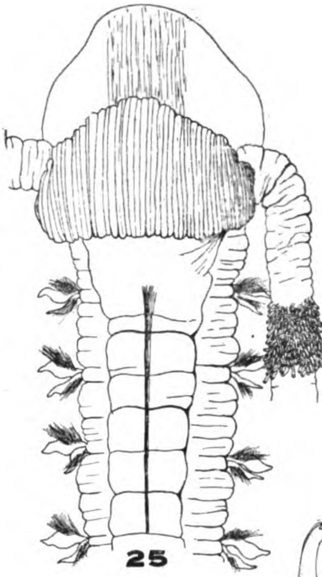
Fig. 33.—A pair of middle notopodial setæ from XV. × 98; *a*, a small portion, × 440.

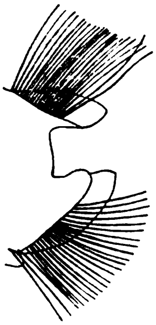
Fig. 34.—An entire crochet from the neuropodium of XL, × 250; and distal end of the same, × 440.

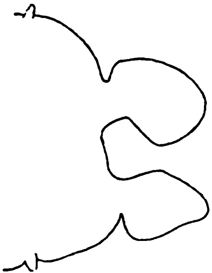


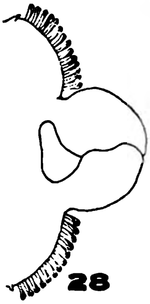
MOORE. NEW SPIONIFORMIA.

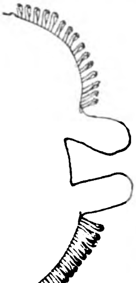

24

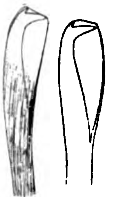

25

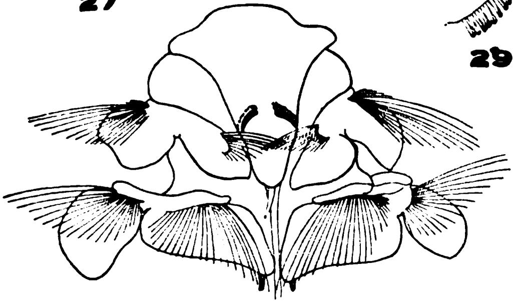

26

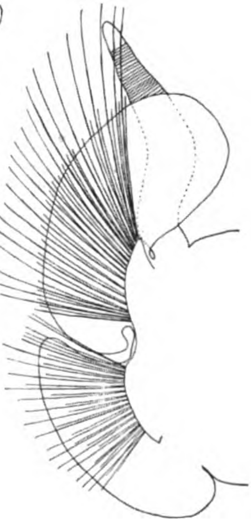

27

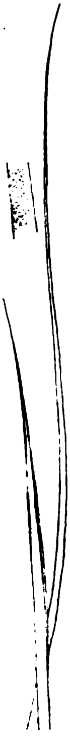

28

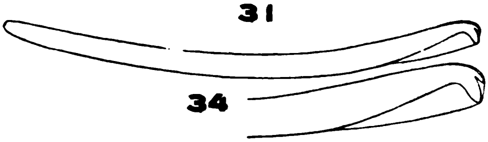

29


30


31


32


33


34

MOORE. NEW SPIONIFORMIA.

THE
AMERICAN NATURALIST

Vol. XL *November, 1906* No. 479

VARIATION IN THE NUMBER OF SEEDS OF THE LOTUS

RAYMOND PEARL

In his *Mutationstheorie* (vol. 1, p. 112) de Vries puts at the head of a list of topics for further investigation by the student of variation the following sentence: "Das Quetelet'sche Gesetz bedarf immer weiterer Beispiele; die Zahl dieser kann nie gross genug werden." In view of this statement from so distinguished an investigator of the problems of evolution, I venture to publish some material on variation in Nelumbium which has been in my notes for some years and which has frequently been used as an illustration in classroom lectures in biometry. As will be seen in what follows, this material conforms very closely to the normal or Gaussian law in the distribution of its variates; much more closely in point of fact than do many cases which have commonly been cited as typical illustrations of that law.

In marshy situations at many points about the shores of the western part of Lake Erie the common lotus, *Nelumbium luteum* Willd., grows in great abundance.[1] Especially in a strip of water known locally as "Black Channel," which connects Sandusky Bay with the lake, does this plant flourish. Many acres of water are literally covered with its leaves. Pieters (*loc. cit.*, p. 66) says of the growth of Nelumbium in this region: "The immense yellow flowers rising just above the great dark-green standing leaves and the water covered with huge floating pads make this the most striking formation of the swamp. The Nelumbium grows in from

---

[1] *Cf.* Pieters, A. J. "The Plants of Western Lake Erie, with Observations on their Distribution." *Bull. U. S. Fish Comm. 1901*, pp. 57–79.

2 to 4 feet of water, or stray plants may be found in less than 2 feet. Many of the floating leaves were 20 to 24 inches across and the standing ones not much smaller. At Upper Sandusky Bay I found a floating leaf 26 inches in diameter and another with a petiole more than 5 feet in length. Both at Sandusky Bay and along the Portage River the acreage of Nelumbium was greater than at East Harbor, but nowhere did the plants present a more vigorous growth or so magnificent an appearance."[1]

The large ovoid seeds of this plant are borne in pockets scattered over the flat, upper surface of the conical seed capsule. After the flower has been shed the ends of the seeds are seen projecting from these pockets. The form of the capsule and the arrangement of the seeds are shown in the accompanying photographs (Figs. 1, 2, and 3), for the preparation of which I am indebted to Miss Frances J. Dunbar.

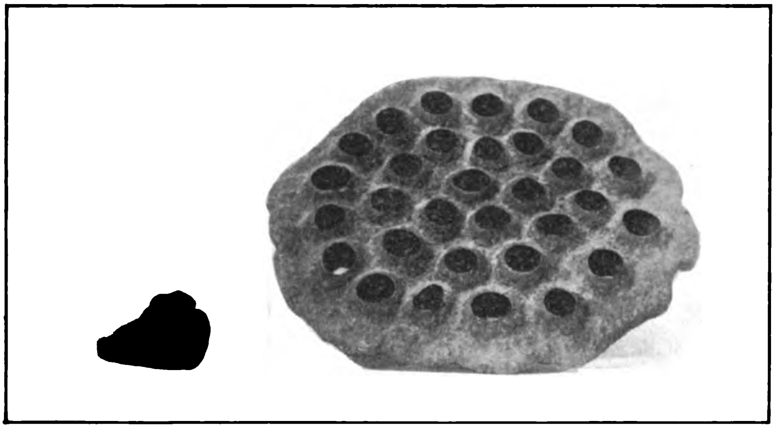


FIG. 1.— Showing the general form of the capsule and arrangement of the seeds in Nelumbium. The two capsules shown in this photograph represent the extremes of variation in the sample: the capsule on the left bore 9 seeds, and the one on the right 39. In the photograph both are reduced below actual size to the same degree.

It is the purpose of the present paper to set forth the results of a study of the variation in the number of seeds to the flower (or the capsule) in this plant. At the end of the flowering season in the summer of 1902 a series of 1410 seed capsules was collected at random from the Black Channel fields in Sandusky Bay. A count was made of the number of seeds in each of these capsules and the records so obtained form the basis of this paper.

The raw data are exhibited in Table 1.

TABLE 1

Frequency Distribution of Number of Seeds in Nelumbium

Number of Seeds per Capsule	Frequency	Number of Seeds per Capsule	Frequency	Number of Seeds per Capsule	Frequency
9	1	20	60	31	45
10	0	21	101	32	34
11	0	22	111	33	21
12	2	23	113	34	13
13	2	24	114	35	11
14	1	25	107	36	7
15	13	26	137	37	2
16	11	27	120	38	1
17	30	28	101	39	1
18	41	29	90		
19	58	30	62	Total	1410

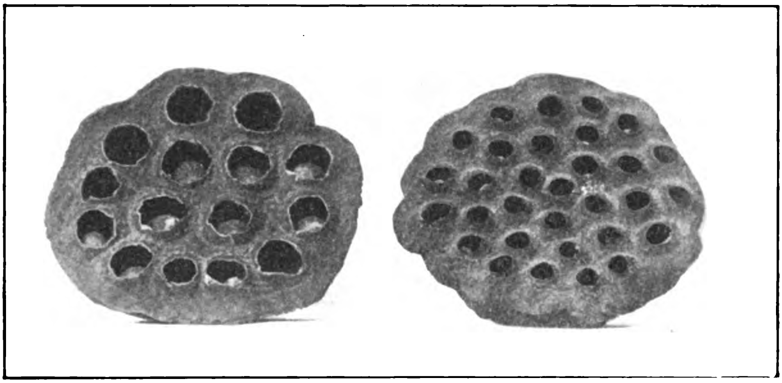


FIG. 2.— Showing two capsules of almost exactly the same size but bearing widely different numbers of seeds, the one on the left having 15 seeds while the other has 31. It should be noted that the openings of the seed pockets have been enlarged with a knife in the specimen on the left. The normal aspect of the capsule top is shown in the right-hand specimen.

The chief physical constants for this distribution are given in Table 2.

TABLE 2

Constants for Variation in Seed Number in Nelumbium

Mean	24.874 ± .078
Standard Deviation	4.339 ± .055
Coëfficient of Variation	17.445 ± .162

It will be noted that the distribution as a whole is quite symmetrical. The relative variability, as measured by the coëfficient of variation, is of the same general order of magnitude as has been found in plant characters by other workers. In order to determine whether or not the variation of the character under consideration follows the normal law within the limits of the errors of random sampling we must examine the values of the analytical constants, which define the character of a frequency distribution, in comparison with their probable errors. Using Sheppard's corrections for the moments I find the values given in Table 3. The unit for the moments is 1 seed.

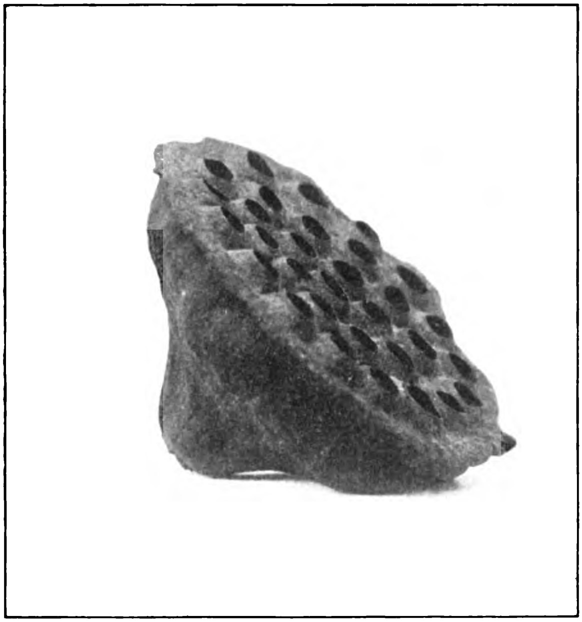


FIG. 3.— A large, fully developed capsule seen from the side.

TABLE 3

Analytical Constants for Variation in Nelumbium

Constant	Value
μ_2	18.8307
μ_3	2.4675
μ_4	1022.5949
β_1	0.0009
$\sqrt{\beta_1}$	0.0302
β_2	2.8838
β_2-3	-0.1162
κ_1	-0.2351
κ_2	-0.0029
Skewness	0.0164
Modal Divergence	0.0712
Standard Deviation	4.3394
Mean	24.8745
Mode	24.8033

Further, we have the following values for the probable errors of the chief constants concerned in testing whether the distribution sensibly deviates from the normal law. It will be understood that these are the values of the probable errors for the normal curve.

Probable error of skewness = ± 0.0220
" " " $\sqrt{\beta_1}$ = ± 0.0440
" " " β_2 = ± 0.0880
" " " modal divergence = ± 0.0955

We see at once that neither the skewness, the difference between the mean and the mode, nor $\sqrt{\beta_1}$, are sensibly different from what they would be for an absolutely normal distribution. In the case of each of these constants the theoretical value for a normal curve is zero. The values found from the actual statistics in this reasonably large sample differ from zero by less than the probable errors. Hence we may conclude that in respect to number of seeds per capsule Nelumbium varies symmetrically about the mean (which of course coincides with the modal) condition. A half of the capsules bear less than the typical number of seeds, and a half more than the typical number. Turning to the quantity β_2-3, which measures the degree of flatness at the top of the curve, or, as it has been called by Pearson,[1] the *kurtosis*, the case is somewhat different. Theoretically the normal curve is mesokurtic, or $\beta_2-3=0$. Now in the present case β_2-3 differs from zero by more than its probable error. The deviation is less than twice the probable error of β_2, so cannot be considered as significant on this basis. As we shall see, however, we get a somewhat better fit to the data given by the *actual sample* if we use a curve which takes into account this deviation from the mesokurtic condition of the normal curve. In so far, however, as we may infer from the sample regarding the conditions in the general population from which the sample is taken, we can conclude with a high degree of probability that *in the variation in number of seeds per capsule Nelumbium follows the normal law of errors.*

From the values of κ_1 and κ_2 given in Table 3 we see that whatever deviation from normality exists, is in the direction of a curve of Type 1. In order to compare the graduation given by a normal and a skew curve, I have fitted both types of curve to the data. The equation to the normal curve is

$$y = 129.6271\ e^{-\frac{x^2}{37.6614}}$$

while the equation to the Type 1 curve is

$$y = 127.6421\ \left(1+\frac{x}{28.7285}\right)^{21.7365}\left(1-\frac{x}{32.1508}\right)^{24.3259}.$$

Calculating out the ordinates of these two curves corresponding to the different numbers of seeds, we have the results shown in Table 4.

TABLE 4

Comparison of Observations and Fitted Curves

Number of Seeds per Capsule	Observed Frequency	Ordinates of Normal Curve	Ordinates of Type 1 Curve
9	1	.2	.06
10	0	.4	.2
11	0	8	.5
12	2	1.6	1.2
13	2	3.1	2.6
14	1	5.6	5.2

---

[1] *Biometrika*, vol. 4, p. 173.

TABLE 4 (*continued*)

Number of Seeds per Capsule	Observed Frequency	Ordinates of Normal Curve	Ordinates of Type 1 Curve
15	13	9.7	9.5
16	11	16.0	16.2
17	30	25.0	25.7
18	41	37.0	38.3
19	58	51.8	53.6
20	60	69.0	70.8
21	101	87.0	88.4
22	111	104.1	104.7
23	113	118.1	117.7
24	114	127.0	125.6
25	107	129.6	127.4
26	137	125.3	123.3
27	120	115.0	113.3
28	101	100.0	99.0
29	90	82.5	82.3
30	62	64.5	65.0
31	45	47.9	48.7
32	34	33.7	34.6
33	21	22.5	23.2
34	13	14.2	14.7
35	11	8.5	8.8
36	7	4.9	4.9
37	2	2.6	2.6
38	1	1.3	1.2
39	1	.7	.6

Of course to get absolute accuracy, areas instead of ordinates should be compared with the observed frequencies, but inasmuch as the number of groups is here large, the error made by comparing ordinates will not be serious.

The frequency polygon and fitted curves are shown in Fig. 4.

The fit is seen to be excellent in the case of both the curves, but the slight superiority of the Type 1 curve is apparent. The difference, as has been pointed out above (p. 762), between this and the normal curve is not significant. The greatest discrepancy between the observations and the curves is in the region about the mode. I am unable to account for the curious irregularity in the

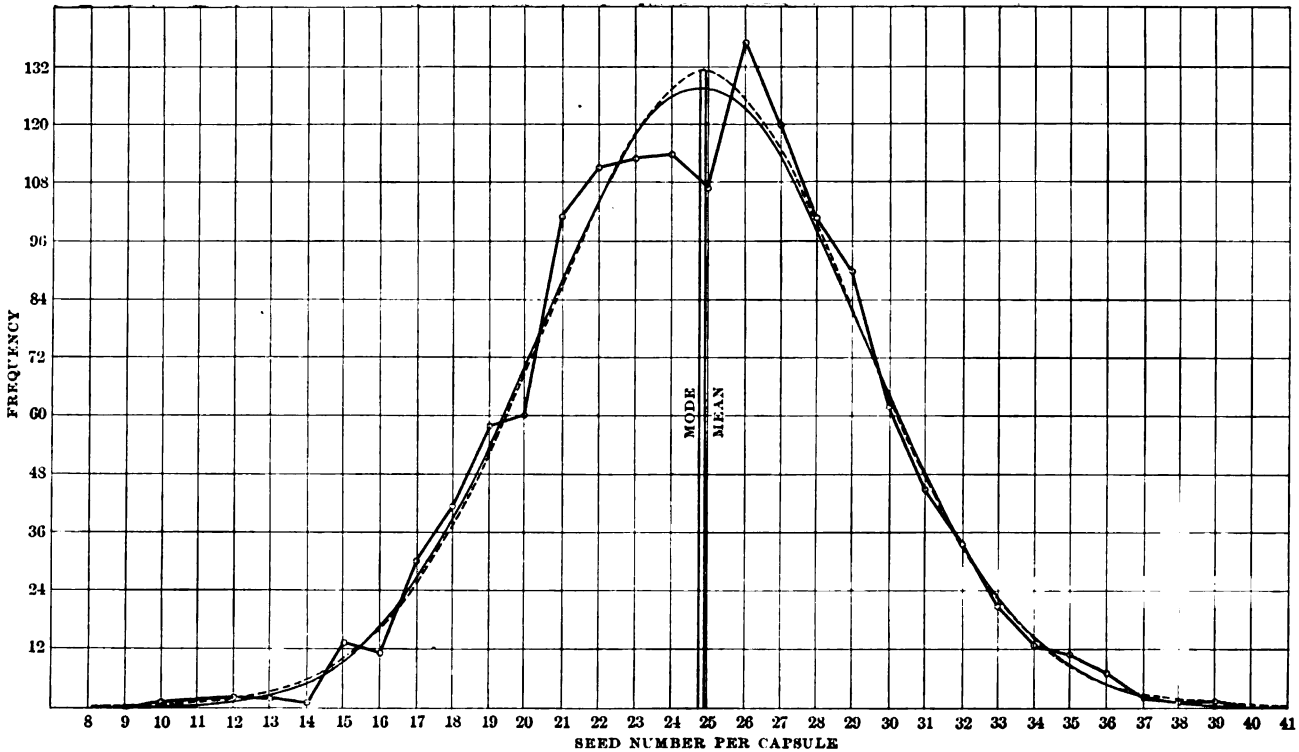


Fig. 4.— Diagram showing variation in the lotus. The abscissæ give seed number and the ordinates frequencies. o——o observations; ----, normal curve; ——, type 1 curve.

observation polygon in this region except as a result of random sampling.

The fact that this distribution approaches very closely to the normal type is indicated by the value obtained for the theoretical range of variation when a Type 1 curve is used. It will be recalled that this type of curve has the range limited in both directions, while the normal curve has an infinite range. Using the values of the moments given in Table 3, I find for the Type 1 curve:—

Total range = 60.8794
Lower limit of range = −3.9252
Upper " " " = 56.9541

It is clear that the theoretical range greatly overestimates the observed. Of course the start at −4 seeds appears at first sight to be an absurdity, but it must be remembered that this value is subject to a considerable probable error, and that it is possible to get as great an extension as this of the range of the theoretical curve below zero as a result merely of random sampling. Furthermore it must be admitted that while the upper limit of the range at 57 seeds seems very improbable, yet, for anything we know to the contrary, it is not impossible.[1] In general it is clear from this case that as the Type 1 curve approaches the normal its range becomes greatly extended.

There is one further point regarding this material to which attention should be called, namely, the bearing of the results on the question of the distribution of fecundity. It is evident that the number of seeds borne by a plant is the measure of its fecundity. In considering data like those here presented the question at once arises as to whether each different class of capsules contributes its proportionate share in the total number of seeds available for the propagation of a succeeding generation. A moment's consideration shows that this cannot be the case in Nelumbium. The figures given in Table 5 demonstrate this. To avoid the possibility of misunderstanding, the manner in which this table is formed may be stated briefly. The figures in the second column were obtained by multiplying the number of seeds in a given capsule by the frequency with which that class of capsule occurred in the sample. The third column gives the same data reduced to *per mille* proportions.

---

[1] Since writing the above I have seen some actual statistics of variation in seed number in the lotus in which the upper limit of the observed range is 42 seeds, showing a tendency in the direction predicted by the theoretical curve.

TABLE 5

Total Number of Seeds borne by Capsules of Different Sizes

Capsule Class (Seeds per Capsule)	Total Number of Seeds borne in all Capsules of Designated Class	Per Mille Number of Seeds borne in all Capsules of Designated Class
9	9	0.26
10	0	0
11	0	0
12	24	0.68
13	26	0.74
14	14	0.40
15	195	5.56
16	176	5.02
17	510	14.54
18	738	21.04
19	1102	31.42
20	1200	34.21
21	2121	60.47
22	2442	69.63
23	2599	74.10
24	2736	78.02
25	2675	76.27
26	3562	101.56
27	3240	92.38
28	2828	80.63
29	2610	74.42
30	1860	53.03
31	1395	39.77
32	1088	31.02
33	693	19.76
34	442	12.60
35	385	10.98
36	252	7.19
37	74	2.11
38	38	1.08
39	39	1.11
Total	35,073	1000.00

From this table we see that, in round numbers, 1400 capsules produce 35,000 seeds. Further, it is clear that the different classes of capsules do not contribute in proportion to their frequency of occurrence to the total seed number. Thus, for example, a reference to Table 1 shows that capsules with 21 seeds each and capsules with 28 seeds each occur with equal frequency in our sample. But obviously the latter will contribute more to the total number of seeds. As a matter of fact the 28-seed capsules contribute 81 per thousand of the total number of seeds, as against 60 per thousand of the 21-seed capsules. Taking the data as a whole I find by a very simple calculation that:

(*a*) Capsules with *fewer* than the median number of seeds bear altogether 15066.325 seeds, or 42.96 percent of the total number.

(*b*) Capsules with *more* than the median number of seeds bear altogether 20006.675 seeds, or 57.04 percent of the total number. In other words 50 percent of the capsules produce 57 percent of the seeds, or, put in still another way, one half of the heads bears 14 percent more of the total number of seeds than does the other half. This result is, of course, an obviously necessary arithmetical consequence of the symmetry of the capsule distribution, yet it is a point which is frequently overlooked. A symmetrical distribution of the individuals of a population with respect to some measure of fecundity does not mean that the contributions of these individuals to the next generation even before selection will be represented by a symmetrical distribution. The very fact that the original distribution is symmetrical necessitates the contrary relation.

The results with reference to the proportionate contributions of the different classes of heads to the total seed number show the conditions before elimination begins. Many of the 35,000 seeds were undoubtedly incapable of germination, and after germination many more would be eliminated before reaching maturity. As to the distribution of the eliminating factors acting in the case of the lotus we know nothing. What I wish to emphasize here is that out of the total number of seeds before elimination begins, 57 percent are the product of one half of the parent heads and only 43 percent the product of the other half.

The results of this study may be summarized briefly as follows:

(1) In the variation in respect to number of seeds per capsule *Nelumbium luteum* follows very closely the normal or Gaussian law of the distribution of errors.

(2) Place constants are given for the designated character in the form unit of Nelumbium growing in Sandusky Bay.

(3) From the fact that the frequency distribution of the capsules in respect to seed number is symmetrical about the mean it follows that one half of the whole number of capsules bears 14 percent more of the total number of seeds available for a new generation than does the other half of the capsules.

Zoölogical Laboratory
University of Pennsylvania
Philadelphia, Pa.

Regeneration in Compound Eyes of Crustacea

MARY ISABELLE STEELE, M.A.

Thesis accepted by the Faculty of the Graduate School of the University of Pennsylvania

MAY, 1907

In partial fulfillment of the requirement for the degree of Doctor of Philosophy

Reprinted from
The Journal of Experimental Zoölogy
Volume V, No. 2
December, 1907

PRESS OF
WILLIAMS & WILKINS COMPANY
BALTIMORE

REGENERATION IN COMPOUND EYES OF CRUSTACEA

BY

MARY ISABELLE STEELE, M.A.

WITH SIXTEEN PLATES AND TWO FIGURES IN THE TEXT

I Introduction.......164
II Methods and material.......165
III The normal adult eye.......168
IV The preliminary regenerative processes.......172
  A Healing of the wound and formation of new cuticle.......173
  B Removal of the injured tissue.......179
  C Method of cell division.......183
V Regeneration occurring after destruction of upper part of eye.......185
  A Regeneration of functional eye.......185
    1 Regenerating eye from surface view.......186
    2 Details of the development of regenerating ommatidia.......190
      a regeneration of retinulæ.......190
      b Regeneration of crystalline cones.......192
      c Regeneration of rhabdoms.......194
    3 Comparison of ommatidia in regeneration and ontogeny.......195
    4 Differences in the regeneration of eyes among Palæmonetes Crangon and hermit crabs.......199
    5 Comparison of normal and regenerating eye.......202
  B Cases of aberrant regeneration of ommatidia.......203
  C Eye stumps that show an abnormal development or no regeneration.......210
    1 Abnormal development of pigment.......210
    2 Eye stumps that show no regeneration.......217
VI Regeneration after removal of the greater part or all of the optic ganglion.......219
  A Hermit crabs.......219
    1 Regeneration of heteromorphic appendages.......219
    2 Cases that show no special regeneration.......221
  B Crangon.......223
    1 Regeneration of heteromorphic appendages.......223
    2 Cases that show no special regeneration.......223
  C Palæmonetes.......225
  D Histology of the heteromorphic appendages.......227
  E General consideration of the regeneration following removal of entire eye.......230
VII Regeneration after splitting the eye longitudinally.......237
VIII Summary.......238
Bibliography.......241
Explanation of plates.......243

I INTRODUCTION

The regeneration of the Crustacean compound eye has been made a subject of observation by a number of investigators. But for the most part end results alone have been described. Very few details of the processes involved in regeneration have been given. To give an accurate description of the histogenesis of the regenerated structures is the chief aim of this paper in the belief that it will contribute something toward a more accurate understanding of the more general problem of regeneration.

The problem has separated itself into three main divisions: first, the regeneration of a functional eye; second, a search for the causes of no regeneration and abnormal regeneration, and third, observations upon the heteromorphic regeneration which may follow the removal of the entire eye.

Herbst ('96, '00) and Morgan ('98) have made the principal observations upon the regeneration of the Crustacean compound eye. But so far as the particular phases dealt with in this paper are concerned Herbst's descriptions are not sufficiently detailed to be of especial assistance in this work. Herbst's observations upon the regenerated heteromorphic structures are somewhat more extensive and in some respects furnish an excellent basis for comparison with the results to be discussed in this paper. For the most part, however, where results have been compared with the work of others the comparison is made between the regenerative and embryonic development and between normal and regenerated structures.

The terminology employed is in great part that used by Parker ('91) in his work upon the compound eyes of Crustacea, it being the terminology in most general use. A few minor deviations have been made but no wholly new terms have been introduced.

The following plan has been followed in this paper. First, a brief description of the normal adult eye is given to furnish a basis for comparison. This is followed by a description of the preparatory regenerative processes which in reality constitutes one of the most important phases of the subject. Then the regenerative processes proper are described under the three main divisions already suggested.

The experimental part of the work was begun at the University of Pennsylvania. Observations upon living material were also carried on during two summers at Woods Hole, Mass., and one summer at Cold Spring Harbor, L. I. The greater part of the detail work of examining the preserved material has been done at the University of Missouri during the present year.

In closing I wish to express my thanks to Dr. E. F. Phillips and Dr. D. B. Casteel for the care of experiments; to Dr. E. G. Conklin, University of Pennsylvania; Dr. C. B. Davenport, Carnegie Institute at Cold Spring Harbor, and Dr. George Lefevre and Dr. W. G. Curtis, of the University of Missouri, for their interest and valuable suggestions during the course of the work.

II MATERIAL AND METHODS

The small hermit crab, Eupagurus longicarpus, the common shrimp, Palæmonetes vulgaris, and the sand shrimp, Crangon vulgaris, afford the greater part of the material used in the series of experiments to be described in this paper. For comparison two species of crayfish, Cambarus virilis and C. gracilis, a species of fresh water Ascellus, the common (wood-louse), Oniscus, and the fresh water Gammarus were used. Other Crustacea also were experimented upon but since no decisive results were obtained they need not be considered here.

The work has been confined chiefly to the eyes of the forms used although experiments upon the appendages, particularly the antennæ, were conducted at the same time. These were, however, largely for the purpose of comparing relative rates of regeneration of the different parts, especially the rate of regeneration of the appendages as compared with that of the eyes.

The experiments upon the eyes consisted in either the removal of a part of the eye or of the whole eye. The part removed varied greatly in the different series of experiments and more or less in individuals of the same series. A limited number of experiments upon Palæmonetes included the removal of both eyes or the removal of one eye with a part of the brain; these operations in most cases resulted fatally. The effect of splitting the eye was also tried upon two series of Palæmonetes, each series was composed of a considerable number of individuals. Results, however, were not particularly different from those obtained after removing a part of the eye.

A total of 600 Palæmonetes and hermit crabs had either one or both eyes operated upon. A much smaller number of Crangon and crayfish were used. No accurate account of the Ascellus, Oniscus and Gammarus was kept. More than 50 per cent of the Palæmonetes and hermit crabs died immediately, or within a short time, after the operation, many of them dying within a few minutes after the eye was injured. Of the survivors about 58 per cent lived through one or more moults.

Forty-two Crangon had the eye operated upon and of these one died of the operation. The crayfish used were for the most part C. gracilis, measuring from 12 to 15 mm. in length, that had moulted but once after hatching.

Palæmonetes and Crangon moult once about every ten days to three weeks. The hermit crabs moult much less frequently, often but once in two or three months. The hermit crabs regenerate, however, as rapidly as Palæmonetes or Crangon.

Considerable difficulty was experienced in keeping the animals alive and in keeping individual records. Finally the plan of keeping each animal in a separate finger bowl was adopted. This method was fairly satisfactory except in very warm weather. Then the water became warm and unless it was changed often the animals soon died. Chopped-up bits of clam were fed to them two or three times a week. Great care had to be used in warm weather for the water became foul, if the food was left in more than three or four hours, and caused the death of the animals.

In spite of all precautions various accidents occurred which resulted in the death of promising material. Twice attempts were made to transfer the experiments from Philadelphia to Woods Hole or vice versa with disastrous results in each case. The failure was due in part no doubt to the extreme warm weather. For although every known precaution was taken most of the animals died within twenty-four hours.

A number of the ordinary fixing fluids were used to preserve material. Among those most frequently used were Fleming's osmic fixative, Perenyi's fluid, Kleinenberg's picro-sulphuric, picro-aceto-sulphuric, Petrunkewitsch's fluid and alcohol acetic. Other fluids also were used and boiling water was tried. The best results were obtained from picro-aceto-sulphuric and Perenyi's fluid. In any case it is difficult to obtain a fixative that does not shrink the inner tissues from the chitin, the regenerated tissues being much more easily affected in this respect than normal tissues.

The embedding was done altogether in hard paraffine 54° to 58° C. melting point. It was necessary to embed the material for a long time in order to cut it without tearing the chitin from the softer tissues. The most satisfactory infiltration was obtained by placing the objects first in equal parts of oil and paraffine, leaving them on top of the water bath over night and then replacing the oil and paraffine with pure paraffine, leaving them on top of the water bath from eight to ten hours longer. Finally they were put in the paraffine bath from one to two hours. Even after the most thorough infiltration it was well nigh impossible to obtain complete series of good sections, because of the difficulty of cutting through the different textures of the material. In dehydrating preparatory to embedding, cedar or bergamot oil was usedin preference to xylol as these oils made the tissues less brittle.

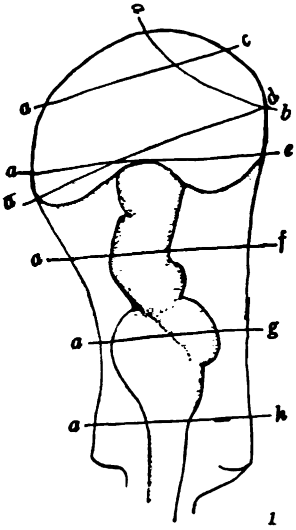
The chief stains employed were Fleming's triple stain and Heidenhain's iron hæmatoxylin. Various counter stains were used with the iron hæmatoxylin but the most generally satisfactory were acid fuchsin and orange G.

In most instances no attempt was made either to soften the chitin or to remove the pigment before the eyes were sectioned. As a rule, however, the material was fixed shortly after a moult so that the chitin was as soft as could be obtained. Any sort of a reagent used to soften the chitin seemed to be more or less injurious to the softer parts, particularly the regenerating tissue. When the chitin was removed, however, 2 per cent nitric acid in 70 per cent alcohol was found to be most satisfactory. It was generally disadvantageous to remove the pigment because this destroyed many of the landmarks both in respect to the regenerating tissue and the condition of the remaining old parts. Sections were sometimes depigmented on the slide. For this Mayer's chlorine method was used.

Table I gives in brief a record of the individuals operated upon, the character of the operation and the end results.

III The Normal Adult Eye

Before taking up the discussion of the regeneration of the eye it will be perhaps well to give a brief description of the structure of



Text Fig. 1 The lines, *a–b*, *a–c*, *a–d*, *a–e*, *a–f*, *a–g* and *a–h* represent approximately the different levels at which a part or whole of the eye has been removed. When the cut was at level as low as *a–e* a part of the optic ganglion was usually involved. In Palæmonetes the eye never regenerated when the cut came as low as the level *a–e*. Hermit crabs may regenerate an eye from the level *a–f* and a heteromorphic appendage from the level *a–h*.

the normal eye. The nature and extent of the operations and the subsequent changes in the eyes will then be more easily understood. The eye of Palæmonetes has been concisely described by Parker ('97) and the following description is adapted from his. It is to be noted that the eye structure of the three forms experimented upon is practically the same.

TABLE I.

PALAEMONETES

Series	No. of individuals	Operation	Date	Died from operation	Lived and moulted	Showed regeneration
I	16	part of cornea removed	May 10	12	4	0
II	12	part of cornea removed	July 11	8	2	1
III	38	part of cornea removed	July 20	29	9	8
IV	40	part of cornea removed	July 28	28	11	8
V	15	part of cornea removed	July 30	3	12	0
VI	16	part of cornea removed	August 1	12	2	1
VII	15	part of cornea removed	August 7	10	3	3
VIII	12	part of cornea removed	November 5	5	5	0
IX	27	part of cornea removed	November 9	8	9	0
I	15	entire cornea removed	March 10	4	7	0
II	18	entire cornea removed	July 20	8	9	1
IV	30	entire cornea removed	July 29	28	2	0
V	30	entire cornea removed	July 30	20	9	0
VI	13	entire cornea removed	August 4	8	5	0
VII	12	entire cornea removed	December 30	11	1	0
		entire eye removed				
I	12	both eyes	January 1	4	6	0
II	12	entire eye	March 5	9	3	0
III	12	both eyes	March 5	12	0	0
IV	18	both eyes	March 10	10	5	0
V	45	entire eye	April 19	12	20	0
I	20	eye split	March 5	5	12	3
I	28	eye split	May 24	16	12	0

HERMIT CRABS

I	12	cornea removed	March 25	2	8	3
II	12	cornea removed	May 26	2	7	2
III	25	cornea removed	July 9	2	16	5
I	15	entire eye removed	May 26	1	12	1
II	25	entire eye removed	July 9	4	14	9
III	8	entire eye removed	October 16	0	3	0
IV	12	entire eye removed	November 27	2	6	0

CRANGON

I	20	part of cornea removed	August 4	0	19	10
I	22	eye removed	August 4	1	19	1

NOTE.—Removing the entire corneal portion of any of the forms was always accompanied by the removal of part of the optic ganglion. In many cases as much as half of it. It frequently resulted that all of the corneal portion subsequently degenerated after a part of it had been removed, and it also frequently happened that the removal of the entire corneal portion resulted in the loss of the whole eye stalk, consequently the above table can be used only as a very general indication of the character of the operation.

The compound eye may be regarded as that part of the optic apparatus contained in the eye stalk. It consists of a large number of ommatidia occupying the distal end of the stalk and a series of four ganglia which extend through the axial portion of the stalk. This series of ganglia for present purposes may be regarded as a compound ganglion composed of four rather distinct sections or ganglionic masses united to each other by nerve fibers. The ommatidia are connected with the distal section of the optic ganglion by the retinular nerve fibers. The optic nerve passes inward from the proximal section of the ganglion to unite the eye with the brain. The basement membrane forms a sort of partition between the ommatidia and the optic ganglion. The transparent chitinous covering over the ommatidial region is known as the cornea.

Each ommatidium is composed of the following cells: two corneal hypodermal cells, four cone cells, two distal retinular cells, eight proximal retinular cells one of which is rudimentary, and a variable but small number of accessory pigment cells. Black pigment granules are contained in both proximal and distal retinulæ and are found only in these cells. The yellowish pigment is confined exclusively to the accessory pigment cells. The different cells enumerated above give rise to the structures that constitute a complete ommatidium. The two corneal hypodermal cells secrete the square corneal facet which covers the outer surface of the ommatidium. Immediately beneath the corneal hypodermal cells is the crystalline cone formed by the four cone cells. The nuclei of these cells are located in their distal ends. The main body of the cone appears as a dense hyaline secretion. Proximally the cone is less dense in structure and tapers to a slender stalk lying between the cone and rhabdom. The rhabdom, according to my observations, is a swollen spindle-shaped structure proximal to the inner ends of the cone cells. The distal retinular cells lie near the inner end of the cone; the proximal retinulæ surround the distal end of the rhabdom. The proximal processes of the retinulæ extend over the rhabdom and pass through the basement membrane as the retinular nerve fibers to enter the optic ganglion below. The accessory pigment cells lie both above and below the basement membrane.

Before closing the description of the normal eye mention should be made of another point. In young individuals in each of the species examined in this series of experiments there is present a growth zone in the ommatidial region. From this zone the number of ommatidia is increased as the animal grows older and increases in size. In longitudinal sections cut in a horizontal plane this zone is apparent on the inner edge of the eye as a narrow band of elongated cells situated above the basement membrane and between it and the completely developed ommatidia. In some instances partially differentiated ommatidia can be recognized in this growth zone. This zone has been mentioned by Parker and others. It is briefly described in this connection now because it is probable that in some instances ommatidia that have apparently regenerated have in reality developed from the growth zone.

The terminology used in the above description and in the discussion of the regenerating eye is that used by Parker. The series of optic ganglia described by him as occupying the eye stalk, however, have been referred to in this paper as different divisions of a single ganglion, it being thought that the matter could be treated with less confusion in this way. Also, the distal portion of the eye stalk, called the retina by Parker, is referred to here as the ommatidial portion. It is composed of a large number of individual ommatidia and the more general use of the term, retina, does not imply all the structures composing the ommatidia. The structure of the rhabdom as described in the present paper is not in full accord with Parker's description.

While the above description applies especially to Palæmonetes it is sufficiently accurate for the other forms described in this work to serve all purposes. The most marked differences in the structures of the eye of Palæmonetes and of the other forms used are as follows: In Crangon and in crayfish the eye stalk is much shorter and proportionately greater in diameter than in Palæmonetes. Also, in Crangon some small glands are found located just below the basement membrane. Hermit crabs have long slender eye stalks similar to those of Palæmonetes except that at the base and occupying the dorsal inner edge there is a small pointed squame bearing a number of sensory hairs. (Fig. 37, *o.sq.*) And finally, there are no accessory pigment cells in the hermit crab's eye. These cells are very conspicuous in the eyes of Palæmonetes.

IV THE PRELIMINARY REGENERATIVE PROCESS

The preparatory stages leading to the regeneration of an eye stump consist chiefly of the following processes; the healing of the wound, the removal of the injured tissues and the active proliferation of new cells of a comparatively undifferentiated character. In removing any part of the eye the injury to the remaining soft, inner tissues is considerable. Especially is this true when the cut passes through the ommatidial region. Much of the tissue surrounding the wound is crushed and torn out of place. On this account the process of healing over the cut surface is much more difficult to follow than the healing of the wound after an antenna or leg has been removed.

Before taking up the description of the preliminary regenerative process it will be perhaps of interest to give in brief the immediate effects of the operation. The death of the animal which so frequently follows close upon the operation seems often to be due chiefly to nervous shock. It cannot be caused by loss of blood alone for usually there is no profuse bleeding. When the eye of Palæmonetes is operated upon the animal often turns over and over ten to forty times as soon as it is released and returned to the water. Many of the animals die before they succeed in righting themselves. Others lie upon their sides several hours after they have ceased revolving and die without showing any normal activities or regaining their equilibrium. It very seldom happens that animals which whirl over and over many times after the operation ever recover from its immediate effects. These apparently helpless motions indicate that the operation has caused the loss of equilibrium.

Crayfish sometimes exhibit these uncontrolled whirling movements. Similar movements are also noted in fresh-water Gammarus and in Ascellus. In Gammarus and Ascellus these movements are executed after the removal of the antennæ or some of the mouth parts. This shows that the effects are not specifically connected with operations upon the eye. Whatever its initial cause the effect is transmitted to the whole nervous system.

In many cases the operation seemed to affect the animal more seriously when only the upper part of the eye was removed than when the entire eye was cut off. Frequently the relative number of survivors was greater in the latter than in the former case. In other instances the animal did not seem greatly affected by the operation regardless of whether the whole or a part of the eye was removed. (See Table I.)

The immediate visible effects upon the eye may be briefly described as follows. As soon as any part of the corneal covering is removed or even as soon as a rent is made in it a considerable amount of the soft, viscous, inner tissue flows out through the opening. It is perhaps carried out by the escaping blood. Much of the pigmented, retinular tissue seems to escape, perhaps because it is softer and more viscous than the other tissues. After an hour or so the remaining, inner tissues are seen bulging out and above the general level of the surface. This is probably on account of the destruction of the normal tension of the tissues due to the changed pressure conditions at the wounded surface. A similar appearance is obtained when the surface injury consists of a rent torn in the cornea with a needle.

A HEALING OF THE WOUND AND FORMATION OF NEW CUTICLE

In a few hours after the operation most of the pigmented tissues have disappeared from the surface of the wound and the swollen surface takes on a whitish appearance. This white swollen surface is apparent for several days. Not until the fourth or fifth day is there any sign of the characteristic red-brown crust which generally forms over wounds in Crustacea.

Sections of an eye fixed six and a half hours after the operation show no definite indications of the healing of the wound. A great deal of the broken and mangled tissue lies outside the wound and hanging to the cuticle about its edges. Inside, the tissues are twisted and misshapen. At the edges of the wound there are slight indications that it is preparing to close over. But the quantity of material lying inside and out makes it impossible to determine what tissues are taking part in closing the wound.

During the next twelve or fifteen hours the changes are still not clearly defined. The interior still presents a rather badly confused mass of injured tissue. Near the edges of the wound, however, there are evidences that the hypodermis has begun to push outward to cover the cut surface. For the most part the wound shows a smooth even surface which indicates that the passage outward of the injured tissues has ceased and that a sort of equilibrium had been established. The mass of tissue closing the cut seems to be made up of a few hypodermal cells and cytoplasmic strands, a considerable accumulation of blood cells and the nuclei of the breaking down tissues of the eye. Around the edges of the cut occasional strands of hypodermis with a very few nuclei can be distinguished.

Sections of an eye fixed about forty hours after the operation show the beginning of crust formation. Almost the whole surface has been covered. Judging by the reaction to stains, the part which may be considered the matrix of the crust is formed by an attenuated, chitinous secretion of the hypodermis. In this matrix are embedded numerous nuclei of the injured tissues together with a great many blood cells and a few hypodermal cells. In some parts the crust is sharply marked off from the underlying tissues by a space filled with coagulated plasma. Over one part of the wound the crust is not yet fully formed. At this point hypodermal strands containing elongated flattened nuclei are seen stretched across the space still uncovered. The strands appear in two or three layers with very few nuclei in each layer. No definite centers of cell proliferation can be recognized at this time.

After the crust has covered the cut surface it continues to increase in thickness for two or three days, then hardens, turns a bright reddish brown color and remains over the stump until a moult has occurred. The crust takes no further active part in the healing and regenerative processes. Fig. 46 represents in a semidiagrammatic manner the crust formed over the wound in a crayfish eye about sixty hours after the operation. The crust is continuous with the inner surface of the cuticle covering the rest of the eye. There are still masses of the injured inner tissues that have been excluded by the formation of the crust clinging to its outer surface. The old tissues in the interior of the eye stump have shrunk back from the crust leaving a considerable space occupied by coagulated plasma. The old cuticle and the matrix of the crust both stain deeply either with orange G or acid fuchsin.

No distinct cuticle can be recognized for several days, from six to eight, after the operation. In the eyes of Palæmonetes that have moulted seven or eight days after the infliction of the injury a cuticle which corresponds approximately in thickness with the cuticle covering the remainder of the eye has formed over the wound. This new cuticle is much looser in texture than the old cuticle. Regeneration does not take place so rapidly in crayfish as in the marine forms examined so that a new cuticle is somewhat longer in forming. Frequently a considerable space intervenes between the overlying crust and the cuticle which has formed beneath it. This is probably due to the recovery of the tissues of the stump from their early swollen condition during which time they were gorged with blood and occupied more than their normal amount of space. It is not unusual to find considerable spaces between different layers of the new cuticle as if a shrinking of the tissues had taken place during the process of forming the new layers of cuticle. The shrinking of the interior tissues without doubt also accounts in part for the folds and wrinkles which often appear in the cuticle over the wound.

The secretion of the new cuticle which grows over the wounded surface begins some little distance back from the cut edges of the old cuticle and is continuous with its inner layers. Fig. 47 is a semidiagrammatic representation of the relation of the old and the new cuticle and the exclusion of the broken down tissue by the development of the new cuticle beneath it. Only eight nuclei appeared beneath the new cuticle in the section as shown in Fig. 47. This figure is taken from an eye that had been injured by tearing through the cuticle with a needle. Reference to the figure shows that very little of the old cuticle had been removed. Fig. 50 shows part of a section near the edge of the wound, Fig. 51 part of a section near the center of the wound. Both figures show the distribution of the hypodermal nuclei beneath the new cuticle that had formed over the cut surface of the eye of a Palæmonetes ten days after the operation. Examination of Figs. 47, 51 will show that a new cuticle may be secreted before a complete hypodermis can be recognized.

Recently hatched Cambarus gracilis, 12 to 15 mm. in length had eyes injured by tearing through the cornea with a sterilized needle. The eyes operated upon in this manner were fixed at different times, varying from eleven to thirty-five days. All of the eyes, however, were fixed before a moult had taken place. In this way it was possible to determine the precise position of the original injury. Sections of such eyes show one point conclusively, at least for Cambarus gracilis. That is that the proliferation of new cells begins from the hypodermis immediately surrounding the rent. From the edges of this proliferating center new cells push out to replace the cells that were removed or that have broken down. Previous observations made upon the regeneration of the eye in crayfish (Steele '04) indicate that crayfish probably do not regenerate a functional eye. It appears, however, that the preliminary regenerative processes are essentially the same in crayfish as in the other forms examined.

It is frequently the case that a much greater proportion of the soft inner tissues are destroyed than of the outer cornea or the hypodermal layer beneath it. When the cut is made the retinulæ and the lower ends of the cone cells press out of the wound and leave the outer ends of the cones and the hypodermis practically undisturbed. Such a condition is particularly noticeable in eyes that were operated upon by tearing the cornea with a needle. In such cases the hypodermal cells *in situ* secrete the new cuticle. This cuticle is, however, without corneal facets, a fact which shows that while the operation neither removed nor caused the disintegration of the hypodermal cells it still affected their activity to such an extent that they no longer function in their usual specialized manner. They now function as the ordinary hypodermal cells over the general surface of the body.

Sections frequently show a morphological transformation of the corneal hypodermal cells *in situ* that are engaged in the secretion of the new cuticle. In the normal adult condition the pair of corneal hypodermal cells that belong to each ommatidium appears as much flattened cells crowded between the distal ends of the cones and the corneal facets. Their nuclei stain faintly and appear to be slender oval bodies lying flat against the cuticle. As the distal ends of the cones in an injured eye break down the nuclei of the corneal hypodermal cells enlarge, become rounded, stain deeply and in every way show signs of increased activity. Fig. 52 includes a series of figures showing the transformation of the corneal hypodermal nuclei into the larger, more deeply staining type seen in the regenerating eye. *a* and *b* in this figure represent the corneal hypodermal cells as they appear in the normal ommatidia. The other figures of this series, *c*, *c′*, *d* and *e*, show corneal hypodermal cells, belonging to ommatidia that have degenerated either wholly or partially. In *c*, *c′*, *e* the distal ends of the cones still remain almost intact and in *c* the nuclei of the corneal hypodermal cells appear but little larger than those associated with normal ommatidia. In *c′* and *e*, however, the nuclei of the corneal hypodermal cells are much enlarged, stain deeply and the cytoplasm surrounding them appears granular and loosely reticular. One nucleus to the left of the figure in *d* appears to be preparing to divide amitotically. That the nuclei shown in *d* are the transformed nuclei of the original corneal hypodermal cells is determined by the fact that on either side of these nuclei are others still associated with partially disintegrated cones. Their original character is also suggested by the fact that they are grouped in pairs. A regenerated, rather than a transformed, hypodermis over the ommatidial region never shows the nuclei arranged in pairs in the early regenerative stages. That the new cuticle has been secreted by these transformed hypodermal cells is shown by the relations of the two structures. The cytoplasmic strands of the hypodermis are continuous with the inner layers of the cuticle (Fig. 52*d*).

Of course it is not absolutely proved that the transformed hypodermal cells take part in the later regenerative processes. This could not be done without examining a very great number of stages. But even the examination of a series of sections from the same eye will show that the indications are strongly in favor of the view that these hypodermal cells remain active and constitute the hypodermis during the subsequent regenerative processes. Fig. 53 is taken from the same eye as *d* in the series shown in Fig. 52. In the later figure it is evident that regeneration is in progress. The hypodermal cells, however, show a tendency toward a paired arrangement indicating that they are the original hypodermal cells.

After a part of the corneal covering has been removed it is evident that an entirely new hypodermis must be regenerated over the wounded surface. In crayfish it was seen that active cell proliferation began near the edges of injured hypodermis and that new cells pushed outward from these centers. The early stages have been examined in a number of eyes of Palæmonetes. The centers of cell proliferation in this form are not so apparent. The nuclei which in the early stages appear beneath the cuticle that covers the wound are very few and lie far apart. Their number increases not chiefly by the repeated multiplication of nuclei at the edges of the wound but by the repeated division of the nuclei that are pushed out onto the wounded surface. During these early stages while the nuclei are actively dividing the cytoplasm is very loose and reticular and the cell boundaries are indistinguishable.

In many cases the new cuticle which becomes apparent after the first moult is somewhat definitely separated into a dense outer portion and a semifibrous inner division. The inner division often appears as an interlacing network of fine fibers, many of which can be traced into the hypodermis beneath (Fig. 53). The loose incompact character of the hypodermis over the wounded surface probably indicates a high degree of activity of the hypodermis in this region.

It is apparent from the foregoing description of the formation of the hypodermis covering the wound that the regenerated hypodermal cells may arise in two ways. They may arise by a transformation of the old corneal hypodermal cells *in situ* in which they assume a less specialized rôle or they may arise by the migration of a limited number of hypodermal cells from the edges of the cut, which later multiply until a complete hypodermis is formed. In either case the first new nuclei must be contributed by the remaining hypodermal cells. Whenever the cut has not removed the entire ommatidial portion the remaining corneal hypodermal cells must assume a somewhat less specialized rôle in order to form the first new nuclei of the regenerated hypodermis.

B REMOVAL OF THE INJURED TISSUE

The fact that the inner tissues of the eye are so much softer than the chitinous outer covering renders it impossible to operate upon the eye in any way or to remove any part of it without serious injury to the remaining softer tissues. It is evident that, before any considerable regeneration can take place, the injured tissues must be either repaired or removed. All the observations made upon regenerating eyes tends to show that none of the injured tissues except the hypodermis ever repair themselves.

Sections of an eye fixed six and one-half hours after the operation show that considerable changes have already taken place in the injured tissues. The effect is particularly noticeable in the retinulæ. Many of the retinular nuclei have become separated from the pigmented retinular processes and appear as rounded bodies surrounded by a dense mass of cytoplasm. These are irregularly scattered among the other tissues. Some parts of the interior have become fairly clear of the broken down structures and are occupied chiefly by coagulated plasma. In other parts of the eye the injured tissues lie in confused masses.

The changes in the next twelve or fifteen hours do not show much advance over the earlier stages. The interior still presents a badly confused mass of broken down tissue. In some parts, however, the cone nuclei appear larger, the bodies of the cones have begun to dissolve and the number of rounded retinular cells appear somewhat more numerous than in the earlier stages, their nuclei showing irregularities in shape (Fig. 75*a* and *b*).

During the earliest stages, six to sixty hours after the operation, the ommatidia that are still intact always appear bent and twisted out of their normal positions. Later stages show that these uninjured ommatidia have regained their original shape and position. This temporary contortion of the ommatidia seems to be due to the immediate effects of the operation, reduction of pressure, destruction of normal tension relations, etc. That in the later stages they appear normal again indicates that they have adjusted themselves to the new conditions imposed upon them by the operation.

Besides those ommatidia that are actually injured by the operation a large portion of those remaining frequently degenerate. The destruction of the tissues of the eye is, consequently, much more extensive than the original injury. This fact is strikingly illustrated by eyes in which the original injury consisted in thrusting a needle into the ommatidial portion. In several such instances the entire, or almost the entire, eye has degenerated. Instances of this kind have been observed in the eyes of several Palæmonetes and also in the eyes of young Cambarus gracilis. Similar phenomena have been observed in eyes of fresh water Gammarus. To be sure the eyes of Gammarus are quite small which may account in a measure for the fact that in six or eight eyes examined in serial sections only one showed any of the old ommatidia intact. In Cambarus gracilis there were two instances out of six in which none of the ommatidial portion remained. In one of the cases the entire eye had degenerated; not even the vestige of the stalk remained. All of this degeneration took place in about thirty days and without a moult. In the other case all of the ommatidial portion and more than half of the optic ganglion had degenerated during the same period. In other eyes of the same series very little degeneration followed the operation. In these extreme cases it seems probable that some infection played a part.

In the degeneration and removal of the injured tissues the retinulæ degenerate most rapidly. In many cases they break down within the first few hours after the operation. Their long pigmented processes become separated from the cell body and collapse into shapeless masses of pigment, which become scattered through the other tissues. The greater part of this pigment finally gathers in clumps near the level of the basement membrane. Although the retinulæ are the first to collapse their remains are the last to be gotten rid of. Evidently the pigment is absorbed and removed only with difficulty. Often in regenerating eyes that have completely regenerated new ommatidia much of the old pigment remains.

The cell bodies of the retinulæ after losing their pigmented processes appear as large nuclei surrounded by a narrow zone of condensed cytoplasm. Within a short time these cells become scattered widely through the eye. After a few days their nuclei appear irregularly shaped and soon afterward become conspicuously polymorphic (Fig. 75*a* and *b*). In the usual course of events these retinular cells disintegrate and disappear. But under some conditions they apparently remain and later multiply and give rise to an abnormal development of tissue that secretes pigment.

The rhabdoms and the inner ends of the cones also degenerate within a short time after the retinulæ. The cones continue to dissolve from the proximal ends distally. The last parts to disappear are the outer ends in which are embedded the cone nuclei. Before the dissolution of the cones is complete the cone nuclei appear greatly enlarged and stain deeply. Their enlarged appearance is probably largely due to the disintegration of the cone substance from about them.

As the disintegration of the tissues proceeds the interior of the eye becomes filled with a granular mass containing scattered nuclei and masses of pigment. This granular mass which is usually very much vacuolated is made up of the remains of the old tissues together with more or less of coagulated plasma and blood cells. Sometimes the remains of the old cones appear as long tapering bands of granular material extending from the periphery inward.

Fig. 51 represents a small area of the disintegrated ommatidial structures as it appeared in the eye of Palæmonetes ten days after the operation. Only two or three nuclei lie close beneath the cuticle and a few others lie scattered deeper in. Most if not all of these more deeply located nuclei are the remains of the old ommatidial structures. In the lower part of the figure are some old pigment remains. The granular mass occupying the greater part of the figure is made up chiefly of partially dissolved cones. The slender strands which can be traced through the granular mass are in part at least made up of new cytoplasm. At certain points the cytoplasmic strands are seen to be continuous with the inner layers of the cuticle.

Fig. 48 is taken from a section of an eye of Palæmonetes seven days after the removal of part of the ommatidia. A surface view from which this figure is taken is shown in Fig. 1. Practically all the material represented in Fig. 48 except the cuticle is made up of disintegrated ommatidia. The long band extending inward shows approximately the position of a former cone. The large vacuolate spaces in the upper part of the figure are old nuclear remains. In the process of disintegration the nuclei at first enlarge and stain deeply. Later the nuclear contents disappear although the nuclear membrane persists for some time longer, often becoming shrunken and folded into a variety of shapes.

It has been seen that the disintegration of the injured tissues begins immediately after the operation and that the greater part is accomplished in from one to three days. The distal ends of the cones alone remain intact for a much longer time, in some cases from two to three weeks and occasionally even longer. The removal of these disintegrated tissues is much slower than their dissolution.

Regeneration proper may and usually does begin within a few days after the old structures have broken down and progresses simultaneously with their removal (Figs. 49, 50). One part of the eye may show ommatidia differentiating while another region is still occupied by disintegrated old structures. The individual differences in the rate of regeneration of such frequent occurrence is probably largely dependent upon the variations in the length of time required for the removal of the old structures. This probably also accounts for the fact that regeneration does not take place uniformly throughout the same eye. The part of the eye that gets rid of the injured tissue soonest regenerates first.

The above observations apply in general to all the forms used. Crangon, however, offers a significant exception in that the injured tissues disintegrate much more slowly than in Palæmonetes, hermit crabs or crayfish.

C METHOD OF CELL DIVISION

In the earlier preliminary stages of the regenerative processes it is impossible to distinguish cell outlines. We should therefore speak of nuclear divisions, perhaps, instead of cell divisions. In later stages the cytoplasm becomes differentiated about the individual nuclei. In all cases of the regeneration of the eye the nuclei are increased by amitotic division. Before a definite hypodermis is established the nuclei can be seen in various stages of constriction, separating off new nuclei for the development of the future underlying structures. There seems to be no perfectly definite manner in which the constriction and separation of a nucleus into two parts takes place. One or two characteristic forms, however, appear so frequently as to be readily distinguished. The two most usual types are seen in the three nuclei occupying the extreme right of Fig. 53. When a nucleus divides in a plane parallel to its long axis it usually assumes the form of the outer one of the three referred to. The formation of the notch on one side gives the nucleus a peculiar heart-shaped appearance which seems characteristic and is easily recognized. Figs. 49 and 53 show nuclei of this same type further advanced in division. The other type referred to is represented by the other two nuclei of the three at the extreme right of Fig. 53. Apparently these two nuclei were recently formed by the longitudinal division of one of the heart-shaped nuclei, like the one lying beside them. Each of these two is now dividing unequally by a transverse constriction.

A dividing nucleolus can sometimes be seen but more frequently a definite nucleolus cannot be distinguished. When nucleoli are seen in dividing nuclei they usually appear with a darkly staining strand of material connecting them. In sections of young Cambarus gracilis eye, nine and sixteen days after the operation, two nucleoli can be recognized in many of the nuclei. In those nuclei that are dividing one nucleolus lies in each part. In all except in the later stages of regeneration of the eye nuclei dividing amitotically can be found in great abundance. But at no time are mitotic divisions seen. In a careful examination of a large number of sections only one cell has been found that suggested the possibility of its being in mitotic division. The appearance of this one suggests a late anaphase or an early telephase. Consequently it is not certain that this is mitotic division. This nucleus appears near the left edge of Fig. 53 at *K*. In any case it must be admitted that amitosis is the regular method of cell division in regenerating eyes of the forms studied, since in every specimen examined during the stages of cell divisions amitosis has been observed and mitosis has not been seen.

That such is the case is somewhat unexpected since Miss Reed ('04) found mitotic division abundant in the regenerating leg of a crayfish. Miss Reed, however, observed that there were no mitotic figures during the early stages of regeneration, although new cells were being rapidly formed. Perhaps we may infer from this that amitosis took place in the regenerating leg of the crayfish during the preparatory stages at least. But one would hardly expect such differences in cell division in forms so closely related for example as crayfish and Palæmonetes. No observations have been made upon histogenesis in the regenerating appendages of hermit crabs, Palæmonetes or Crangon. Hence it cannot be said whether or not the eye furnishes an unique exception to the regeneration of other parts in these forms.

Recently, however, many have come to regard amitosis as a phenomenon of more frequent occurrence than has been generally supposed. Meves ('91 and '94), McGregor ('99), Child ('04 and '07), all describe amitosis as a normal phenomenon and consequently no longer accept Vom Rath's view that a cell is nearing its final dissolution when it begins to divide amitotically.

On the other hand many of the more conservative investigators are unwilling to admit that amitosis occurs as a normal phenomenon and believe that the apparent cases of amitosis can be explained on some other grounds. But in all the instances hitherto described amitosis has been found occurring along with mitosis. In the present case, however, all of the cell divisions are amitotic and they all take place in cells derived from the hypodermis; in these respects the regenerating eyes investigated in this series of experiments offer a unique instance in their method of cell division.

V REGENERATION OCCURRING AFTER DESTRUCTION OF DISTAL PART OF EYE

Under this heading will be discussed the results obtained from eyes injured in varying degrees but not exceeding the destruction of more than the distal two sections of the optic ganglion. The injury originally inflicted varied from tearing the surface of the cornea with a needle to cutting off the whole top of the eye so that at least the first part of the second section of the optic ganglion had been removed. Frequently, however, as has been explained in Section IV the part of the eye ultimately lost was much greater than that originally removed. For in cutting the eye with scissors or tearing it with a needle much of the tissue surrounding and underlying the wound was so injured that it afterward degenerated.

In the series of experiments to be described under this division nearly two hundred Palæmonetes, twenty Crangon and fifty hermit crabs were used. Fifty per cent of the Palæmonetes, 5 per cent of the Crangon and 10 per cent of the hermit crabs died of the operation. Of those that survived the loss of blood and the nervous shock of the operation a considerable number died from other causes without having undergone a moult. Forty-five Palæmonetes, seventeen Crangon and twenty-nine hermit crabs, however, lived through at least one moult. Among these a number showed no regeneration either from surface examinations or from sections. The greater number of those that gave surface indications of regeneration and for comparison a number that showed no regeneration have been sectioned and examined.

A REGENERATION OF THE FUNCTIONAL EYE

Experience has shown that the number of days an experiment covered serves to indicate only in the most general way the stage of regeneration. While the time element naturally constitutes a most important factor the rate of regeneration is also dependent upon the season of the year, the age and the physiological activity of the individual and perhaps other factors not so apparent. Besides individual differences displayed between members of the same species there also appeared to be differences in the ability to regenerate and the rate of regeneration among the three species chiefly used, these differences being much more marked in the regeneration of an eye than in the regeneration of an appendage. The hermit crabs seem to completely regenerate an eye in shorter time than either Palæmonetes or Crangon. But the final results were similar in all three forms. The few significant differences will be pointed out and discussed later.

1 Entire Preparations of Regenerating Eyes

A careful examination of the regenerating eye frequently reveals a number of important features and is absolutely essential to the later interpretation of the sections. Therefore outline surface drawings have been made of all eyes later sectioned. In most cases the normal eye was drawn in connection with the regenerating eye for comparison as to size, shape, etc. Frequently the two eyes were sectioned together. These surface views which had better be regarded as optical sections were drawn with a camera after the eyes had been brought into oil preparatory to embedding. Figs. 1 to 25 represent various stages of regenerating eyes.

Fig. 1 shows dorsal and ventral views of a Palæmonetes' eye seven days after an operation which removed the ventral corneal surface and immediately after a moult. A comparison of the dorsal and ventral views shows that the injury is confined chiefly to the underside. In shape the eye is practically normal. It is not even flattened on the underside as the thinning out of the pigment near the center would seem to indicate. As is usually the case the injured eye is much smaller than the uninjured one. Not only the region operated upon but the whole eye decreases in size after the operation. This indicates that however localized the operation may be the effect is much more extended. In this particular case the part of the eye proximal to the injury measures only three-fourths of the length of the same region in the uninjured eye.

Fig. 3 represents the ventral view of a Palæmonetes eye ten days after being injured. In this case also the injury extends across the ventral side of the ommatidial portion. The pigment of the broken down ommatidia can be seen scattered in flakes and patches through the upper part of the eye. From the dorsal side the eye appeared nearly normal but sections show that almost the entire eye is in process of degeneration.

The specimen represented in Fig. 4 shows a regenerating eye nineteen days after the removal of almost all of the ommatidial portion. It is readily seen that very little remains except the eye stalk. The pigment patches are remains of the original eye. Across the end of the stump the cuticle is wrinkled and folded, indicating that comparatively little new tissue has been formed and that the cuticle follows more or less closely the rough uneven outlines of the wounded surface.

When the entire ommatidial portion has been removed or has degenerated regeneration seems to be considerably slower than when a large part of it remains uninjured. The two specimens shown in Figs. 9 and 10 afford a striking illustration of this fact. Both of these eyes were operated upon at the same time. Each animal moulted twice, the first time on the same day and the second, a day apart. Both were fixed in picro-acetic at the same time, thirty-two days after the operation. In Fig. 9 the injury involved only the posterior ventral side, less than one-half of the ommatidia. While in Fig. 10 the injury included all of the structures lying distal to the basement membrane. Examination of the sections shows new ommatidia completely differentiated in Fig. 9 while in no part of Fig. 10 are they yet defined.

The next specimen, Fig. 20, presents a rather striking appearance and suggests immediately that the regeneration taking place is not altogether of the normal type. This is a thirty-eight day specimen and belongs to the same series of experiments as the preceding two specimens. All of the part distal to the dark pigmented band is regenerated tissue. The pigment consists chiefly of the remains of the old retinulæ. It is evident even from a surface view that no ommatidia have developed. Sections show, however, that on one side the differentiation of cones is beginning (Fig. 69). Here it may be well to mention a fact that has been observed a number of times. The regeneration of the new ommatidia never presents a uniform stage of differentiation in any case whether or not all of the old ommatidia have disappeared. In fact it may be possible to select several stages from the same eye and sometimes two or three stages from the same section.

An eye thirty days after the injury, Fig. 6, shows some interesting features in comparison with the one just described. From a ventral view this eye shows no signs of remaining ommatidia and from the dorsal side only a very few cones and facets are evident. The upper part of the eye is transparent. Below this transparent area are scattered patches of pigment representing the remains of the old eye. Sections show that no new ommatidia have been entirely differentiated, that the retinular cells have differentiated and are establishing connections with the optic ganglion, that new cone nuclei are being separated from the hypodermal nuclei, that on one side a few of the old ommatidia remain and that in the tissues lying nearest the old ommatidia new cones are being developed. The most striking feature presented by this specimen is the clearness with which the connections between the retinulæ and the ganglion cells can be made out. These connections will be discussed at length in the consideration of the detailed structures of the regenerating eye.

Fig. 17 shows the ventral view of a regenerating Palæmonetes eye from a thirty-five day specimen. Examination of the dorsal side shows that a large number of the ommatidia on that side appear uninjured, the greater part of the injury being confined to the ventral side as indicated by a surface examination. Sections show, however, a gradation of regeneration from a stage in which there is no signs of cone differentiation to the complete formation of a new ommatidium.

It was said above that hermit crabs regenerated an eye more rapidly than either Crangon or Palæmonetes even in instances where a considerable portion of the optic ganglia had been removed. Figs. 5, 12 to 15 show regenerated eyes of hermit crabs after one or more sections of the optic ganglion have been destroyed. Fig. 12 had at least the distal division of the ganglion removed. The regenerated eye shown in the figure was developed within thirty-three days and after only one moult. This moult occurred on the thirty-second day and the eye was fixed in Perenyi the thirty-third day. Sections show that ommatidial structures have been fully differentiated although incompletely developed. One point is particularly noticeable in these sections; the ommatidia are very much shorter than in the normal eye. This condition was probably caused by the mechanical pressure of the covering cuticle which forced the developing ommatidia into less space than they would otherwise have occupied. Fig. 13 shows the eye of a hermit crab regenerated from a stump in which not more than half of the optic ganglion remains. The regeneration in this eye took place in thirty-eight days. One moult occurred twelve days after the operation. Although extremely small the eye is practically perfect except that the corneal facets have not yet developed.

Figs. 14 and 15 show two other regenerated eyes of hermit crabs forty-one and sixty-seven days respectively after the operation. Sections of the forty-one day eye do not show ommatidia as fully developed as the thirty-eight day specimen previously described. The sixty-seven day specimen shows the eye complete in all its details even to the corneal facets. Whether a younger regenerated eye might not show the corneal facets has not been determined since no specimens were available between the forty-one day and the sixty-seven day specimens.

A noticeable feature in all the regenerated eyes of the hermit crabs is their small size in comparison with the normal eyes. It is probable that the regenerated eyes would have increased in size if the experiment had covered a longer period of time. Sections of the eye shown in Fig. 12 indicate that the definitive size has not been reached. For, lying outside the fully formed ommatidia are others in the process of development. In the case of the eye shown in Fig. 13 sections do not show any indications of partially developed ommatidia and it may be that this eye would never have reached the size of the normal eye. Perhaps this is what we should expect since the amount of nervous tissue present is considerable less than is normal.

It is more difficult to interpret the actual condition of a regenerating eye in Crangon than in either Palæmonetes or hermit crabs. The whole of the eye stalk in Crangon is thickly covered with branching pigment cells. So that even after the eyes are brought into oil very little can be seen in detail by examining them in toto. Figs. 7 and 8 thirty-one and thirty-two days, respectively, are fairly characteristic of the regenerating eye of Crangon. From all that can be determined from the outside, regeneration seems practically complete in each of these cases. Sections show, however, that comparatively little regeneration has taken place. A fuller discussion of the regenerating eye of Crangon will be taken up elsewhere.

2 *Details of Development of the Regenerating Ommatidia*

The complete regeneration of the ommatidial portion of the eye involves three stages. These can be separated from each other rather sharply although they overlap more or less. The first stage consists in getting rid of the broken-down tissues and the healing of the wound; second, the active proliferation of new cells; and third, the differentiation of the new ommatidia. The first and second stages have been discussed in Section IV.

a Regeneration of Retinulæ

All the observations support the conclusion that the regenerated ommatidia are derived entirely from the hypodermis. Before the hypodermis covering the end of the stump has been clearly differentiated, however, the proliferation of cells for the new structures lying below has begun. So that at the same time hypodermal cells are dividing in two planes, one at right angles to the periphery to increase the number of hypodermal cells, and the other parallel to the surface. The inner nuclei of the latter division migrate inward and become the first retinular cells. As they migrate they become elongated with their long axes radially arranged.

Fig. 54 shows the early stages of the separation of retinular nuclei, some separating from the nuclei at the periphery and others migrating in. At a comparatively early date these retinular nuclei have migrated a considerable distance below the surface and may be seen in a relatively well defined row (Fig. 68). Soon after the retinular nuclei have been separated from the nuclei at the surface they themselves begin to divide (Fig 68) in a plane at right angles to their plane of original division. These longitudinal divisions may begin before the nuclei have reached their definitive position. This division continues until a band composed of many nuclei has been formed. Figs. 60 and 62 show portions of such bands.

Here and there single nuclei are found lying much nearer the basement membrane than the retinular band (Figs. 60, 62). These are occasionally seen constricted. It has been impossible to determine with certainty the fate of these scattered nuclei but there are evidences which suggest that they become the nuclei of the accessory pigment cells.

The retinular nuclei even in the early stages of their migration stain much more deeply than the nuclei at the periphery. But at this stage no definite cytoplasmic outlines can be distinguished. Very faintly staining delicate strands of cytoplasm, however, can be found extending between the retinular nuclei and the periphery. These strands form an intermingled network and with the nuclei lie in a granular substance. Soon after the nuclei have reached their definitive position the cell bodies of the retinulæ can be recognized. Each nucleus appears surrounded by more or less definite strands of cytoplasm which are radially arranged and extend outward toward the periphery and inward to the basement membrane (Figs. 60, 65). These can now be definitely recognized as retinular cells. Usually it is easier to see the proximal than the distal strands for at an early stage these lower processes begin to secrete pigment and are consequently more conspicuous (Fig. 65). At the stage represented by Fig. 62 there is only the merest beginning of pigment deposition. The fibers are but little differentiated from their background which still seems to be composed largely of a homogeneous granular material, probably to a great extent coagulated plasma.

At a stage such as is represented by Figs. 60, 62, in which there is only the merest beginning of pigment secretion delicate cytoplasmic processes can be traced from the retinular nuclei inward through the basement membrane beneath which they can be found branching over the ganglion cells. Fig. 62 shows several isolated retinular cells and their proximal processes which are seen extending through the basement membrane and reaching to the ganglion cells beneath. There is no evidence that these retinular fibers are directly connected with the ganglion cells; they seem merely to twine around them.

When the fibers have reached the basement membrane they may pass directly through it as the two shown in the left side of Fig. 62, or they may extend along the upper face of the basement membrane before entering the ganglionic mass below. The retinular processes frequently branch shortly before entering the basement membrane or just as they emerge below it. No special nerve methods were employed yet numbers of these fibers are seen branching among the ganglion cells and many are readily traced from the retinular nuclei to the basement membrane (Fig. 65). It is only in very favorable specimens that the fibers can be traced, however, through their whole length.

b Regeneration of the Crystalline Cones

Up to and including the stages shown in Fig. 65 and described above there is no evidence of any differentiation of the crystalline cones. A definite hypodermis, however, has been formed with an increased number of nuclei, from which other nuclei are separating. These are the cone nuclei (Fig. 63).

The formation of the cone nuclei does not appear to take place in a uniform manner. The usual method, however, is by the division of the hypodermal nuclei in a plane parallel to the periphery, the inner nuclei thus formed being the cone nuclei. But since in every ommatidium there are four cone nuclei and only two hypodermal nuclei, it is evident that either the hypodermal cells must divide twice or the first cone nuclei must themselves divide in order to make the cone nuclei just twice the number of the hypodermal nuclei. Observations indicate that in some cases the second pair of cone nuclei arise by the division of the first pair. In other cases, however, it is uncertain whether they arise in this manner or whether they arise from the hypodermal nuclei. It is probable, however, that they are formed by the division of the first pair of cone nuclei as can be determined in some cases.

In the early stages of cone formation the inner surfaces of the hypodermal cells lose their distinctive outlines. For at this time there is no clear line of demarcation between the hypodermal and cone cells. Fig. 63 represents one of the earliest recognizable stages in crystalline cone formation. The hypodermal cells are more or less definitely grouped into pairs and it is readily seen that cone and hypodermal nuclei are not wholly separate. Extending inward from the cone nuclei are very delicate strands of cytoplasm. These strands seem to group the hypodermal nuclei into pairs and by their branching and crossing form a much vacuolated network.

At a stage slightly more advanced (Fig. 64) the cytoplasm of the cones has begun to assume a more definite cone shape. There is still, however, no distinct line of separation between the hypodermal and cone nuclei. Neither does the cone mass show the boundaries of its component cells. In stages a little later the cone cells begin to show individual outlines and the cytoplasm appears more condensed. Cone formation is practically complete, however, before the corneal hypodermal and cone cells show a distinct line of separation (Fig. 66).

As the cone cells differentiate the cytoplasm becomes less and less vacuolate and gradually assumes a dense granular appearance. The cytoplasm is most condensed just below the nuclei and decreases in density proximally.

In longitudinal sections of the cones the cell boundaries appear distinct from their outer ends inward to the outer retinulæ. At this point the cell boundaries become indistinct and the cone tapers rather suddenly into a slender stalk which extends to the distal end of the rhabdom, where it ends abruptly (Fig. 66). At a somewhat more advanced stage the boundaries between the cone and corneal hypodermal cells become distinct, and the cone secretion takes on the dense homogeneous deeply staining appearance characteristic of that in mature cones (Fig. 67).

c Regeneration of the Rhabdoms

There is no indication that the retinular cells have begun to secrete the rhabdoms until after the cones have been distinctly outlined, although the retinulæ themselves become clearly differentiated before there is any indication of the cones. Not until the differentiation of the ommatidia has reached a stage intermediate between these stages shown in Figs. 64 and 66 can the anlagen of the rhabdoms be recognized. The rhabdoms first appear as slender homogeneous rods. Each rod is of uniform diameter throughout its length, and is distinguishable from the inner ends of the cone cells only by the fact that it stains slightly deeper and shows no divisions which indicate that it is composed of more than one cell (Fig. 66.) The rhabdoms show no signs of the characteristic spindle-like form and the complicated system of transverse plates so noticeable in the normal adult eye until after the last stage in the differentiation of the cones (Fig. 67). Even at the stage shown in the preceding figure the rhabdom does not show a normal appearance of its spindle form and the pigmented extensions of the retinular do not cover it so completely as in normal adult ommatidia.

It is evident from the preceding description and accompanying figures that all the structures necessary to a completely regenerated eye have been laid down. It is also seen that with the exception of the corneal facets the regenerated ommatidia are practically identical with those of the normal adult eye. A specimen of later stage, however, shows both the corneal facets and the definitive form of the rhabdom, so that the regenerated ommatidia present a perfectly normal appearance even to the minutest detail (Fig. 76).

All observations show that the differentiation of corneal facets could not become evident until after at least two moults. The first cuticle which is developed is formed before a continuous hypodermis has grown over the wounded surface and before any regeneration of ommatidia has begun. Corneal hypodermal cells are not differentiated as such until all of the other ommatidial structures have been laid down. Therefore the secretion of corneal facets constitutes the final process in the regeneration of ommatidia. Since this is true, several moults may occur before the corneal facets differentiate, and at least two must take place.

3 Comparison of Ommatidia in Regeneration and Ontogeny

In comparing the regenerating with the embryonic eye it is necessary to consider them only from the beginning of ommatidial differentiation, since there can be no exact parallel between the preliminary stages of regeneration and the mode of the origin of the embryonic eye. These two processes are similar, however, in the respect that the ommatidia in both cases develop from the hypodermal cells. All these observations upon the regenerating eye give evidence that the cells which take part in the formation of the new ommatidia are derived primarily from the hypodermal cells that cover the wounded surface. That the ommatidia of the embryonic eye develop entirely from the hypodermis is the conclusion of most observers. There is no further agreement, however, in details except in the case of embryonic eyes that are described as arising without invagination, *e. g.*, the compound eye of the honey bee as described by Phillips ('05) and of the lobster (Parker '90), in which it was found that the ommatidia are developed from a single epithelial layer and consequently from morphologically similar cells.

It has been seen that the first cells differentiated from the hypodermis in the regenerating eye are the retinular cells. This can be regarded as being in agreement with the conclusion of Phillips that the retinulæ constitute the morphological center of the ommatidium. At any rate the retinulæ are in each case differentiated before the cone cells can be recognized, none of the cells originally separated from the hypodermis to form the retinulæ ever take any part in the formation of cones, and finally the cone cells arise peripheral to the retinulæ.

The differentiation of the regenerating ommatidia, described in a preceding section, and of the embryonic eye, as described by Kingsley ('87), may perhaps be regarded as presenting a parallel. Kingsley finds the nuclei which go to make up the cones and retinulæ arranged in radial rows and that the outer and hence the later formed nucleus of each row contributes to the formation of a cone while the remaining nuclei form the retinulæ.

Unlike the rows of nuclei described by Kingsley and the spindle shaped groups of cells described by Phillips the retinulæ of the regenerating eye do not appear to become separated into definite groups before the development of the cones. In sections from the same eye there may be groups of retinulæ somewhat distinctly separated from each other and other retinulæ which constitute a continuous band for a considerable distance. Figs. 60 and 61 illustrate these opposite cases. But even when groups can be recognized there is no certain indication that a group belongs to a single ommatidium. The group may contain a fewer or a greater number of cells than belong to a single ommatidium. Besides the retinulæ continue to divide occasionally up to the time the cones are differentiated. From the evidence furnished by a number of different specimens it appears that the definite separation of the retinulæ into groups does not take place until after the cones are well advanced in their development. As the cones differentiate from the periphery inward the retinulæ become grouped about them. As this grouping continues the retinular processes become more and more slender, perhaps largely as a result of mechanical pressure.

The development of the cone as shown by my observations is the result of intra-cellular secretion. In this respect it agrees with the embryonic development of the cones in the eye of the honey bee as described by Phillips. It is directly opposed to the method described by Patten ('87), Kingsley ('87) and Watase ('89), who regard the cones as the result of the extra-cellular secretion of the cone cells.

The evidence furnished by the regenerating eyes of Palæmonetes, Crangon and hermit crabs agrees with the observations of those who do not find the cone and the rhabdom to be developed in the embryonic eye as continuous structures. Some investigators regard the rhabdom as merely an inward prolongation of the cone cells. Kingsley finds such a relationship in the embryonic development of the eye of Crangon. Patten regards the cone as extending from the hypodermis to the basement membrane and as differentiating at the lower end into the rhabdom in most cases. But in Vespa he describes the inward prolongation of the cone cells as enclosing the rhabdom. Parker ('00) describes the prolongation of the cone cells in Homarus as extending to the basement membrane and inclosing the rhabdom in the same manner. The description of the relation of the rhabdom to the cone in Crangon, given by Kingsley, and applied to Crustacea in general by Patten, does not agree with the facts presented by the regenerating eyes of Crangon, Palæmonetes and hermit crabs. Obviously, however, this interpretation is in accord with that of Phillips in the case of the honey bee and that of Grenacher ('74), both of whom find that the rhabdom is developed as a secretion of the retinulæ, and do not find the cone cells extending as slender processes beyond the distal end of the rhabdom.

Concerning the source and manner of the innervation of the ommatidia the results obtained in this study of the regenerated eye agree only with those observers who, like Parker ('91) and Phillips ('05), regard the retinular cells as hypodermal sense cells which send nerve fibers into the ganglion below. It is true that no special nerve methods were used in this work upon the regenerating eye. But in some specimens at least the prolongation of the retinular processes into fibers which penetrate the optic ganglion is clearly evident (Fig. 62). In many other cases the processes can be traced from the retinular nuclei to the basement membrane and similar processes are found branching among the ganglion cells below it. But in no case is there the slightest evidence that the ganglion cells are sending fibers upward to the regenerating ommatidia. Consequently there seems to be no room for reasonable doubt that the retinulæ form the nerve endings of the ommatidia.

In this particular these results differ from those of Patten, Kingsley and other workers on the embryological development of the eye of certain Decapods. These investigators regard the nerve connections as being formed by the extension of processes upward from the ganglion cells, through the rhabdom and into the cone.

The observations made in this work seem neither to uphold nor to oppose the views of those who find that the ganglion cells send processes upward into the retinulæ during the embryonic development. For it is not inconceivable that the innervation of the ommatidia of a normal eye should be accomplished by the upward growth of processes from the ganglion cells to the retinulæ and that in the regenerating eye it should be accomplished by processes growing inward from the retinulæ to the ganglion cells. That this is not impossible is suggested by the fact that in regeneration tissues are sometimes developed from the same germ layer while they arise from different germ layers in embryonic development.

Several instances are known where muscles in regenerated appendages arise from the hypodermis although normally they are of mesodermal origin. Miss Reed ('04) finds this to be true in the regenerating leg of the crayfish. Ost ('07) notes the same phenomenon in the regenerating antennæ of Oniscus.

It is recognized, then, that certain tissues originating normally from different germ layers may arise in a regenerating organ from the same germ layer. It would be at least possible that, although the nerve connections between the optic ganglion and the retinulæ arise as processes from the ganglion in the development of the normal eye, they might arise as processes from the retinulæ in the regenerating eye. As even in this case they would develop from the same germ layer although from different parts of it.

The possibility that the nerve connections may have arisen differently in the embryonic eye and in the regenerating eye is conceivable. Yet it seems that the evidence obtained from a comparative study of the normal adult eye and the regenerating eye suggests that the nerve processes develop from the retinulæ in the normal eye just as in the regenerating eye.

The preceding pages show that the development of the regenerating compound eye corresponds in a general way with the embryonic development of the compound eye. They also show that the observations made upon the regenerating eye do not agree entirely with those of any one worker upon the embryonic development of the compound eye of Arthropods. In many respects, however, there is a close similarity between the development of the regenerating eyes of Palæmonetes and hermit crabs and the process of differentiation in the embryonic eye of the lobster as described by Parker (*loc. cit.*) Further, these observations upon regenerating eyes agree with those of Phillips upon the developing compound eye of the honey bee in regard to the order of appearance of the retinulæ and cones, in the method of innervation of the ommatidia and in regard to the relation of the cones and rhabdoms. In the developing eye of the honey bee, however, Phillips finds the rhabdoms partially differentiated before there is any indication of the cones. On the other hand in the regenerating eyes of hermit crabs and Palæmonetes the cones are definitely formed before any rhabdoms can be recognized. The variations, however, which have been noted between the developing compound eye of the honey bee and the regenerating eyes of Palæmonetes and hermit crabs, cannot be regarded as fundamental. Such differences are perhaps not more marked than those that would be noted if the embryonic development of the same eyes were compared.

It is scarcely necessary to add that these observations on regenerating eyes are in several respects quite at variance with the observations of Kingsley and Patten, who find the rhabdoms developed from an inward prolongation of the cone cells, the cones formed as extracellular secretions and the ommatidia innervated by nerve processes coming from the optic ganglion and penetrating the rhabdoms and cones.

4 *Differences in the Regeneration of the Eye Among Palæmonetes, Crangon and Hermit Crabs*

Reference has already been made to the fact that certain differences in the regenerating eye appear among Palæmonetes, Crangon and hermit crabs. The rate of regeneration and the ability to regenerate varies greatly in these different genera although in the most essential particulars the regeneration of the ommatidia is similar. It has been seen that hermit crabs may regenerate an eye after the removal of half the optic ganglion. But neither Palæmonetes nor Crangon regenerate a perfect eye if the injury includes any part of the optic ganglion. It has also been seen that the differentiation of the ommatidia takes place more rapidly in the hermit crabs than in either of the other forms. The only structure in the eye of the hermit crab which apparently does not regenerate perfectly is the basement membrane. This membrane, however, is but slightly developed in the normal eye. It is not strange, therefore, that it appears imperfect in the regenerated eye.

The regenerated eyes of the hermit crabs in these experiments have developed from a level below the basement membrane. In every case they present a clearer and more normal appearance than the regenerated eye of either Palæmonetes or Crangon. This is largely due to the fact that in the eye of the hermit crab there are no shapeless masses of old pigment scattered among the regenerated tissues as is usually the case in Crangon and Palæmonetes. The absence of the yellow accessory pigment cells in the eye of the hermit crab also tends to give to the ommatidia a distinct and orderly arrangement. The absence of this accessory pigment is, however, not due to incomplete regeneration. The normal eye of the hermit crab, unlike that of many Decapods, contains no accessory pigment cells. These pigment cells are very abundant in the eyes of Crangon and Palæmonetes and tend to make the ommatidia less clear.

The most significant difference between Palæmonetes and Crangon seems to be in the rate of regeneration after similar injury. External appearances would indicate that Crangon regenerates more rapidly than Palæmonetes. But a comparative study of the section shows that the reverse is true.

In almost every individual in the series of twenty Crangon operated upon the injury was slight. The wound healed rapidly, the animals moulted frequently and externally there was every indication that regeneration was rapidly taking place. An examination of the sections, however, shows that in none of them has there been any considerable regeneration. On the other hand, in each case much of the old injured tissue remained in a semi-broken down condition.

Sections of the eye represented in Fig. 8 show that one part of the eye had not been injured below the level of the outer retinular cells. The proximal ends of the retinulæ still remain intact although thirty-one days have elapsed since the operation. A continuous hypodermis has not yet been formed. A considerable area between the cuticle and the outer retinulæ is occupied by a granular structureless mass. Just to one side of this area is a region in which none of the old ommatidia appear but in which there are new ommatidia almost completely formed. No more than five of these appear in any one section. These lie near the basement membrane toward the inner edge of the eye. Consequently these new ommatidia lie next the growing zone, always present in the eyes of young individuals as described in Section III. It is apparent then in this particular case that it is impossible to determine conclusively whether the new ommatidia are regenerated or normally developed ommatidia. In other cases, however, new ommatidia are found developing in positions where it is evident they are regenerating ones.

From the evidence obtained by an examination of a number of regenerating eyes of Crangon it seems that the rate of regeneration depends largely upon the rate of removal of the injured tissue. The failure of the old tissue to degenerate prevents the regeneration of new structures. Since the injured ommatidia, although they fail to break down for a considerable time at least, are incapable of regeneration in themselves. We should perhaps expect the cones to be incapable of any sort of regeneration for the cone nuclei have been destroyed and the constructive metabolic activity of a cell apparently depends largely upon the nucleus. The nuclei of the retinulæ, however, have not been injured and still retain much of their normal appearance. But there is no evidence that the retinulæ ever take part in normal regeneration.

A comparative study of the regenerating eyes of Palæmonetes and Crangon shows that the difference in the rate of regeneration is in reality largely a difference in the rate of degeneration of the injured tissues. In Palæmonetes the injured tissues usually break down rapidly and are quickly removed. In Crangon they persist indefinitely. Hence regeneration in Palæmonetes begins soon after the injury, and new ommatidia may be almost fully developed in shorter time than is required by Crangon for the removal of the injured ommatidia. The specific case of Crangon perhaps suggests that if all of the ommatidia had been completely removed by the operation, that regeneration would have followed more rapidly. An inference which is supported by the observations of Zeleny ('05), who finds that in regenerating appendages of crayfish, an increase of the injury, increases the rate of regeneration. On the other hand, the same inference cannot be applied to the regenerating eyes of Palæmonetes; for as has already been pointed out for this form new ommatidia differentiate more rapidly when a part of the old ommatidia remain uninjured.

5 Comparison of Normal and Regenerated Eyes

It seldom happens that a regenerated eye appears altogether normal either from external or internal examination. Externally they frequently appear abnormal in shape and are always smaller than the opposite eye. These external abnormalities are, however, of no especial importance except in so far as they may indicate internal conditions. A common external feature which is suggestive of internal conditions is the irregular arrangement of the pigment masses. Internal examination shows that these masses are frequently remains of broken down retinulæ.

Besides the pigment remains of the old retinulæ, a number of other abnormalities may appear which make it difficult to interpret sections correctly. The arrangement of the retinulæ makes it difficult to group them into distal and proximal rows of nuclei as can be done readily in the normal eye. It is quite possible in cross sections to select ommatiia in which eight retinulæ, the typical number, can be counted but in other cases this number cannot be recognized owing possibly to the suppression of the eighth retinular cell, which is rudimentary in the normal eye. The difference in the length of normal and regenerated ommatidia is quite noticeable in many cases. The regenerated ommatidia are often much shorter. The new ommatidia might have grown to normal size, however, had the experiment covered a longer period of time.

The remaining significant difference between the normal and regenerated eyes is that in some regenerated eyes the optic ganglion is not complete. This difference appears only in the hermit crabs as these forms may develop an eye after half the optic ganglion has been removed. In no case is there any evidence that the optic ganglion regenerates. Consequently this difference would remain unchanged.

B CASES OF ABERRANT REGENERATION OF OMMATIDIA

In study of the regenerating eye several cases of aberrant regeneration of ommatidia have come under observation. One case deserves especial mention. Fig. 36, *a* and *b* represent ventral surface views of an injured right eye and the normal left eye of a Palæmonetes. Judging both from the surface indications and an examination of the sections the right eye must have been cut off at a level corresponding approximately with the line *a–b* shown in the figure of the normal eye. A cut at this level would remove the upper two sections of the optic ganglion and injure the third. It would also cut across the heavy muscle band lying in the posterior part of the eye stalk.

The experiment covered thirty days, one moult taking place ten days after the operation. Casual surface examination was sufficient to show that a rather large amount of new tissue had formed and that a spot of pigment had developed on the ventral side of the stump. The growth of such a large amount of new tissue is quite unusual when so much of the ganglion has been removed.

A study of the sections gives additional information regarding this new tissue. In Fig. 56 the tissue lying between the periphery and the broken line extending from *x* to *y* represents approximately the amount of new tissue. Careful examination shows a difference in the character of the differentiation of the regenerated tissues in the different regions. This figure is from a section so near the dorsal surface that but little of the nerve tissue appears. Near the right side of the figure a conspicuous section of the old muscle band is seen. Just distal to the muscle band the new tissue is more dense and compact than in the remaining part of the regenerating tissue. This band of new tissue (*nt*) is composed of fibers extending inward from the periphery and joining end to end with the fibers of the old muscle band, thereby forming a continuous band and reëstablishing the broken connection between the muscle and the chitinous covering of the stalk.

Fig. 57 of the same series represents a section deeper in from the dorsal surface so that parts of the optic ganglion are apparent. A few scattered spots of pigment are also present. Here again the regenerated tissue shows differentiation into strands of fibers in the part lying beyond the remains of the old muscle band. In other regions there is a loose network of fibers with scattered nuclei. A difference, however, in the appearance of the nuclei in different regions of the regenerated tissue can be observed. From *a* to *b* the nuclei are small and inconspicuous, constituting uniformly granular masses and staining with but little more intensity than the fibers which extend inward from them. The nuclei lying between the points *b* and *c*, on the contrary, are conspicuous, stain deeply and are more than twice the size of those lying between *a* and *b*. Here also a number of nuclei are seen lying below the periphery which show a tendency to extend straight inward from the periphery. Fig. 58 shows the upper part of a section that lies so near the ventral surface that it is entirely outside most of the optic ganglion. In this figure no part of the muscle band appears. Conspicuous masses of pigment are present in this section as well as a great number of relatively large deeply staining nuclei. Since the eye was cut longitudinally from the dorsal to the ventral side the sections near the ventral side are approximately tangential, so that many of the nuclei that appear to be deep in from the periphery are in reality near to the surface. This needs to be kept in mind in interpreting the figures.

It is a conspicuous fact that many of the nuclei shown in Fig. 58 resemble in shape and appearance the retinular nuclei found in sections of regenerating eyes. Further resemblances between these and retinular nuclei are their tendency to stain deeply and the general direction of their long axes which is at right angles to the surface. These facts considered in relation to each other leave but little doubt that these elongated nuclei represent the retinular elements in a regenerating eye. At one point in Fig. 37 (*c.c.*) the rudiments of two crystalline cones have appeared. This is additional evidence that an eye is regenerating, imperfect and abnormal as it may be. The pigment shown in this figure represents the maximum amount seen in any section. For the most part it presents no definite arrangement but lies in irregular masses clustered within a fairly well defined area. In a few sections, however, a part of the pigment shows a tendency toward a normal arrangement as if the pigment granules were contained within the processes of the retinular cells, and rudimentary ommatidia can be recognized (Fig. 59).

Any attempt to explain the phenomena presented by the eye under discussion may appear somewhat premature, since it presents a practically unique case so that but little data for comparison is available. In the first place this is the only well established case of any attempt of Palæmonetes to regenerate ommatidial structures after the removal of a large part of the optic ganglion. It seldom happens that any regeneration takes place from the eye stump of Palæmonetes when no more than half of the optic ganglion remains. Before attempting to explain the phenomena, therefore, it is well perhaps to examine the results of other observations that may suggest an explanation.

From the evidence obtained from the study of normally regenerating eyes the indications are that the first new regenerated tissue is largely of an indifferent character, *i. e.*, capable of giving rise to different structures, as determined by conditions more or less external to itself. It has been seen that in the regenerating eye the primary hypodermis gives rise to the cells which develop into the different structures of the ommatidia. In the earliest beginning of differentiation, if a cell divides so that the plane of division is at right angles to the surface the two resulting cells are hypodermal cells. If on the other hand the plane of division is parallel to the surface the inner one of the pair thus formed becomes a retinular nucleus and the outer one remains hypodermal in character. At this stage the only apparent difference between the two nuclei is in their respective positions. In later cell generations when the division plane is parallel to the surface the inner nuclei of the pairs formed become crystalline cone nuclei. Thus we have cone nuclei, retinular nuclei and hypodermal nuclei indistinguishable except for their relative positions. Apparently the subsequent differentiation of each is conditioned by their relative positions in respect to each other, to the surface and to the old parts present.

That the relation of the old tissues to the regenerating tissues is a determining factor in the regenerating of the new structures has been maintained by several workers. Child ('04) who especially emphasizes the idea, says: "The fate of the new material must be regarded as depending essentially upon its relation to the old parts." That the regeneration of one structure may be dependent upon the presence of another has been shown by Lewis ('04). He found that a lens could be developed from any part of the ectoderm of a frog embryo by transplanting the optic vesicle and allowing it to come in contact with the ectoderm. In this case it appears that the actual contact of the two tissues constituted a determining factor and that the new conditions have arisen on account of the new relations of the two tissues.

From the instances referred to the following inferences may be drawn—first, newly regenerated tissue is largely indifferent in character; second, the differentiation of the new tissue is largely conditioned by its relation to the old tissue. The above inferences may be used in suggesting an interpretation of the special phenomena under consideration.

To begin, it is evident that the particular individual now being considered exhibited a more than usual degree of physiological and regenerative activity. In no other way could we account for so much more new tissue than is ordinarily regenerated by a stump of this length. The sections show that a large part of the new tissue still presents an undifferentiated appearance, although in certain regions differentiation has begun. Just above the cut end of the old muscle band (Fig. 35) the new tissue appears thickened, arranged in definite fibers and is apparently continuous with the muscle band suggesting that connections between the muscle and the chitinous covering of the eye had been reëstablished.

Sections passing through the stump near its ventral side show rudimentary ommatidia in process of development. Just why ommatidia should appear on the side of the old stump is not at first sight apparent. One possible explanation of this phenomenon has, however, suggested itself. The differentiation of new ommatidia appears to depend largely upon the reëstablishment of connections between the optic ganglion and the new tissue. That such is the case is suggested by the fact that in regenerating eyes the retinular processes reach the optic ganglion before the cones begin to differentiate and before any pigment is deposited in the retinulæ. Further, according to Parker (*loc. cit.*) in the embryonic development of the lobster's eye from the earliest beginning of differentiation there is a connection between the ommatidial region and the optic ganglion and this connection is never lost at any stage in the development of the eye. Since the pigment appears in the retinulæ after they have formed connection with the ganglion its presence in this stump (Fig. 36a) may be an indication that the connections between the optic ganglion and the new tissues have been formed and consequently that further development of ommatidial structures has been initiated.

This suggested explanation does not of course give any reason why ommatidial development should begin on the ventral side rather than elsewhere. The following explanation is suggested. Cross and longitudinal sections of the normal eye stump show that the optic ganglion extends somewhat nearer the surface toward the anterior ventral side. On this account perhaps rudimentary ommatidia have developed on the ventral side first because the distance between the new tissue and the optic ganglion was shorter so that nerve connections were more quickly established in that region than elsewhere.

Other cases of aberrant regeneration have also come under observation. These, however, have been produced apparently by pathological conditions. One or two of the more interesting cases will be described. Fig. 19 represents dorsal surface views of a Palæmonetes eye thirty-one days after the removal of the upper part of the ommatidial portion. Eight days after the operation a moult occurred. A second moult occurred fifteen days later and it was seen that the greater part of the ommatidial structures had disappeared. Fig. 19 shows a mass of pigment just distal to the optic ganglion. Above this pigment there is a considerable area of transparent tissue which shows no external evidence, however, of being differentiated into ommatidia. Sections of this eye show a considerable development of abnormal pigment. At one side are seen a limited number of regenerating ommatidia that have failed to differentiate normally. The arrangement of the abnormal pigment is of a character frequently seen in short stumps but not generally found where the injury, as here, has not involved the optic ganglion. The probable cause of this abnormal pigment deposition will be considered in another section. The point of chief interest here is that we find two sorts of development going on side by side, one region developing normal structures and a contiguous region developing abnormal structures.

Another similar, though rather more exaggerated, case is furnished by a small hermit crab (Fig. 25). The eye had been injured before the animal was brought into the laboratory. No moult, however, had taken place since the injury. About two weeks after being brought into the laboratory the crab moulted and three days later was killed. Fig. 25 represents the ventral view both of the injured and uninjured eye three days after the moult. It is seen that the injury involved the whole ommatidial portion and apparently a part of the optic ganglion. The distal surface of the regenerating eye presents a very irregular outline. On the inner border a peculiar protuberance has developed and two separate pigment areas are apparent. The upper of these two areas suggests that small ommatidia have been regenerated. Externally the lower pigment mass suggests no probable explanation of its character.

Sections of this eye show a number of interesting points. In the first place it is demonstrated that the upper and smaller pigment area belongs to the retinulæ of small but almost completely developed ommatidia. All the structures of a typical ommatidium have been differentiated with the exception of the corneal facets and the spindle shaped enlargement at the base of the rhabdom. The lower pigment area is an abnormal pigment deposition similar to the preceding case.

The protuberance developed on the inner border of the eye seems also to be formed of abnormal tissue. Its interior is entirely made up of a loose irregular network of tissue containing a number of faintly staining nuclei. These tissues resemble very closely the depigmented tissues of the abnormal pigment masses. This resemblance suggests that possibly the two abnormal appearances have had a common origin.

A case partially resembling the one just described was observed in a green shrimp, Palæmonetes viridis. The original injury consisted in the removal of a small part of the top of the eye. The eye was operated upon August 1. On the ninth of the same month the animal moulted and was preserved. Figs. 17 and 18 represent dorsal and ventral views of the injured eye. The dorsal surface of the whole eye is shown in Fig. 17. Fig. 18 represents the distal end of the eye from the ventral side and under greater magnification. A pigmented mass similar in general outline to the retinular area in the normal eye is visible through the transparent outer tissues. Distal to the pigmented portion is a considerable area of transparent tissue with flecks of pigment scattered through it.

The distal contour of the eye is irregular because of a swelling or protuberance similar to the one on the hermit crab's eye previously described. This eye also shows an unusual development of new tissue considering the time in which it was produced.

Sections of this eye show that the optic ganglion had not been injured, that not all of the ommatidia had been removed and that a considerable part of the old pigment remained. The ommatidia that were left have almost completely degenerated, however, and the whole distal portion of the stump is filled with a complicated network of faintly staining cells. There is also absolutely no regularity in cellular arrangement, as is seen in normally regenerating eyes. For the most part the nuclei are scarcely distinguishable from the cell-body. Although here and there are scattered nuclei which stain more deeply. There are evidences in some cases that these are nuclei of disintegrating ommatidial structures. Some sections show remains of old cones associated with the darkly staining nuclei. Comparison of others of these deeply staining nuclei with the nuclei of partially depigmented cells shows a similarity between the two which suggests that the former belong to cells in which pigment secretion has lately begun.

It is evident that most of the pigment masses present are the remains of the old ommatidia although they are greatly scattered through the new tissue. A few dense cysts of new pigment, however, have been formed and other pigment secreting centers have begun to appear. From these observations it seems apparent that had the animal lived the entire mass of tissue sooner or later would have been densely packed with pigment cysts and that very probably new eye structures would not have regenerated. For we have seen in the preceding two cases that an abnormal secretion of pigment stopped, apparently, ommatidial regeneration after it had begun. It does not seem too much to assume then that in this case normal regeneration of tissues would have been precluded by such an abundant development of abnormal tissue.

C EYE STUMPS THAT SHOW AN ABNORMAL DEVELOPMENT OR NO REGENERATION

The instances described in the preceding section apply particularly to those unusual types in which the ommatidia have begun to regenerate and this process has been more than balanced by opposing factors. This leads naturally to a consideration of cases in which there is either no regeneration or only an abnormal development of pigment.

1 Abnormal Development of Pigment

Most of the examples of abnormal pigment secretion were afforded by Palæmonetes in which the optic ganglion was more or less injured. Usually in any of the forms studied eye stumps that contain no more than half of the optic ganglion show no normal regeneration aside from the cuticle and hypodermis. Any attempt to regenerate other tissues produces either scattered strands of connective tissue or abnormal masses of pigment. These pigment masses most frequently appear collected in nodules or cysts and are usually enclosed in a sort of connective tissue sheaths. Fig. 43 represents an eye stump of Palæmonetes, showing one of these pigment depositions. Fig. 72 shows an outline section through this stump from which the relation of the pigment to the normal issues can be readily made out. Fig. 43 shows in detail the appearance of the pigment outlined in Fig. 72. An examination of Fig. 73 shows that the deposition of pigment appears to begin at several centers. These centers gradually increase in size. There also seems to be a tendency for the several centers to fuse with each other. It is further seen that the pigment cells or masses vary from very large to very small areas.

A study of depigmented sections suggests that these smaller pigment bodies arise in one of two ways: first, by an out-pocketing of the cytoplasm, which after becoming distended with pigment separates from the parent mass and second, by an unequal division of the cell. It is possible that the latter is the true method for all cases. But it was not possible to determine this point with certainty. When a pigment cell has become gorged with pigment the nucleus is much changed and distorted. And even after the most thorough depigmentation it cannot always be identified. Consequently it may be that the smaller masses, in which no nuclei are visible, are not mere masses of cytoplasm that have been constricted off but are the result of unequal cell division. Fig. 75*f* shows a small group of depigmented pigment bodies. In the larger masses nuclei are visible. In the smaller bodies nuclei cannot be determined with certainty.

The amount of pigment within a cell varies. Some cells contain only a few scattered granules while others are so completely filled that they appear to be black homogeneous masses. In these more densely filled cells the pigment appears to have fused into solid brittle masses that can be crushed like starch grains. *g* in Fig. 75 represents one of these masses after it has been crushed.

The pigment is dissolved from the sections with the greatest difficulty. Mayer's chlorine method was generally used for this purpose. But in removing the cyst-like depositions of pigment it was found that alternate treatment with the chlorine method and with one-twentieth per cent KOH in 70 per cent alcohol gave equally as good and more rapid results. Even with this treatment twelve to twenty-four hours were required to remove the pigment from sections 6μ thick. Frequently this failed to dissolve the dense pigment masses. In Fig. 75 the dense, crushed pigment mass *g* lies in the same section with the group of depigmented cells shown in *f*. It frequently happens that not all of the tissues included in what may be regarded as a single pigment region are pigmented (Fig. 73). The unpigmented tissues, shown in the area represented in this figure, contain but few recognizable nuclei. Here and there are cells that show a few pigment granules and occasionally small groups of such cells. These facts together with the general appearance of the tissue suggest that eventually the entire area might have become packed with pigment.

Figs. 29, 32, 44 represent eye stumps of Palæmonetes that show somewhat different types of these abnormal pigment formations. Fig. 32 presents a rather unusual type. Externally the pigment appears as thickly scattered granules instead of a dense black mass as in most cases. Sections of this stump show a small quantity of new tissue lying at the distal end and alongside the nerve stump. The cells composing the new tissue are closely packed, large, granular, and their nuclei do not take up iron hæmatoxylin at all. Along the side of the eye stump a number of small pigment cysts appear but for the most part the cells of the new tissue are not yet densely pigmented. Many of them, however, show numerous pigment granules. This particular specimen shows less of the connective tissue-like, fibrous network than is usually found in the pigment areas. Apparently this stump shows an early stage in abnormal pigment secretion. The other two cases figured show dense masses of pigment. Fig. 44 presents a single compact mass. In each case sections show the pigment arranged in the characteristic cysts, such as are seen in Fig. 73.

One additional fact of interest is shown in Fig. 44. The pigment cysts in this case do not lie wholly above and distal to the remains of the optic ganglion but are embedded in the end of the optic stump. Apparently the upper part of the ganglion stump has degenerated and given place to the pigment. This is not an unique instance as several other stumps have presented a similar phenomenon. There was one case in particular in which there were several small pigment cysts embedded in different portions of the remains of the optic ganglion. The ganglion, in this case had almost entirely degenerated, apparently. This animal had been preserved in alcohol, however, and it was consequently impossible to determine just how much of the abnormal appearance of the tissue was due to degeneration before the death of the animal and how much was due to disintegration after its death.

It is evident that this sort of pigment development, whatever may be its cause, does not belong to the normal regeneration of an eye. Further, it appears probable that the causes leading to its formation are of such a nature that they inhibit the true regenerative process. The last two cases described in the preceding section furnish evidence of this. In the eyes shown in Figs. 19 and 20 regeneration of normal ommatidia had begun but was limited by some opposing factor. These causes not only inhibit the true regenerative processes after they have begun but it is also probably true that they even prevent true regeneration from beginning. All the comparative evidence that we have indicates that in the case of the Palæmonetes viridis previously described (Fig. 17) a new eye would never have developed. The whole distal end of the stump was filled with a mass of abnormal pigment depositing cells. Although this case is striking it is not exceptional. Similar conditions have been found in varying degrees in other eye stumps. There is sufficient similarity in all the cases of abnormal pigment deposition to indicate that they have in certain respects a common cause.

It is important to point out some of these similarities in greater detail. A striking resemblance exists between the broken down retinulæ of an injured eye and the pigment secreting cells. In the early stages of the disintegration of the ommatidial structures the nuclei of the retinulæ frequently become separated from the retinular processes. Each nucleus becomes surrounded by a rounded mass of cytoplasm which apparently has no connection with other structures. The nuclei become polymorphic and not infrequently appear divided. As the disintegration proceeds these rounded nuclear cells usually disappear, but, as mentioned in a preceding section, the broken down masses of pigment remain. These rounded remains of the retinulæ can be identified from a few hours up to sixteen days after the injury. They are always seen a few hours after the injury although they may not always be present in eyes examined in a week to two weeks after the operation. This shows that in some cases they disintegrate much more rapidly than in others. In some eyes examined twenty-five to thirty-five days after the operation similar rounded cells with polymorphic nuclei are found in numbers, increasing by amitotic division. In still other cases, cells of this character containing pigment granules are found.

Fig. 75*a*, *b*, *c*, *d*, *e*, *f*, *g* represents a series of groups of these rounded cells with polymorphic nuclei. These groups were taken from crayfish, hermit crabs, Crangon and Palæmonetes, representing in all seven species. The first three groups, *a*, *b* and *c*, show the appearance of breaking down retinulæ, seventeen hours, thirty-nine hours and sixteen days, respectively, after the injury; *d*, *e* and *f* show the secretion of abnormal pigment as found in eyes ten, twenty-three and sixty-seven days respectively after the injury; *f* represents a group of depigmented cells that were so filled with pigment that without depigmentation no structures were visible. An examination of this series cannot fail to show the similarity between the breaking down retinulæ and the pigment secreting cells. Particularly is this so if it is remembered that, except *a* and *b*, no two groups are taken from the same species.

These facts taken together have suggested that the immediate cause of the pathological pigment secretion is the abnormal activity of old retinulæ which have not completely broken down. It has already been mentioned that after an eye has been operated upon the pigment from the injured retinulæ frequently becomes greatly scattered among the other tissues. Not only does the retinular pigment become scattered but in some cases the rounded retinular cells, also, are found considerable distances down the stalk and on the side opposite the injury. These instances were observed in eyes examined from fifteen to twenty days after the injury. It seems probable that some of these metamorphosed retinular cells become embedded in other tissues, then later divide amitotically and begin to secrete pigment. The nodules of pigment previously described are the result. In some cases the multiplication of these pathological cells takes place rapidly so that large areas are occupied by them. Fig. 70, which is from a section of the eye shown in Fig. 20, represents such a case. A relatively large amount of new tissue was regenerated by this eye, and sections show that normal regeneration had begun (Figs. 68, 69). A new hypodermis was completely differentiated and on one side the differentiation of ommatidia was taking place (Fig. 69). The greater part of the new tissue was made up, however, of cells of a character known to be abnormal. The hypodermal cells are practically the only cells that appear normal. With the exception of a small area on one side almost the whole of the interior is filled with rounded cells containing polymorphic nuclei. Fig. 71 represents a part of Fig. 70 more highly magnified and showing the structure in greater detail. A comparison of these two figures with the series shown in Fig. 75 cannot fail to show a striking similarity.

Altogether there is strong evidence that the failure of the old retinulæ to disintegrate completely is the immediate cause of abnormal pigment deposition, in many cases at least. Further, there is some evidence that regenerating retinulæ may sometimes become involved in the abnormal secretion of pigment. Group *d*, Fig. 75, represents a probable case of this sort. The group was taken from the regenerating part of the eye in a region where there is positive evidence that some normal regeneration is taking place. A few of the cells in the group still show but few pigment granules and show elongated nuclei, characteristic of regenerating retinulæ (Fig. 75*d*, *ret.n.*)

The appearance of the network of tissue with which many of these pigment nodules are associated still remains to be accounted for. Evidences which point to the origin of this are not so numerous as are the evidences that the old retinulæ form the centers for the pigment secretion. In some cases these pigment nodules are found embedded in the hypodermis of the eye stalk, in other cases in the membrane surrounding the optic ganglion. In such instances it seems probable that the fibrous network supporting these nodules is due to the hypertrophy of the normal tissue immediately surrounding the pigment deposits. In those cases, however, where a great mass of this fibrous network developed it seems to have had a different origin. There are three particularly striking instances of the unusual development of this abnormal tissue, each furnished by a different form. Sections taken from a crayfish eye fixed sixty-two and a half hours after the operation show a condition that is apparently an early stage in such development. At this stage the network is not yet compact and is found in chains of elongated cells, showing nuclei dividing amitotically. These chains of cells run in all directions but do not appear to develop from the hypodermis. Some sections show these chains extending from the injured retinulæ which still surround the remains of the old cones (Fig. 74). This suggests that the old retinular cells are undergoing rapid multiplication.

The chains of cells found in the crayfish eye differ in the following respects from the network of abnormal tissue found in Palæmonetes viridis and hermit crab, shown in Figs. 17 and 25. In the cases of the hermit crab, Fig. 25, and of Palæmonetes viridis, Fig. 17, the cells constituting the network are no longer recognizable as chains and the nuclei no longer stain deeply nor appear to be dividing. These differences may be accounted for by the following facts. First, the whole available space in the stump of the eye was completely filled with the network in the eyes of hermit crab and Palæmonetes viridis and the chains of cells had become so completely interwoven that their original character could no longer be recognized. Second, it is probable that the nuclei no longer stain deeply because the cells have ceased active division. It has been shown that the cells cease to divide actively soon after the secretion of pigment begins. In these cells the secretion of pigment had begun.

Assuming that these apparent differences have been accounted for we may now turn to their likenesses which suggest a similarity of origin. The most suggestive likeness is that in each of the three forms the abnormal tissue appears to have developed outward from the base of the wounded area rather than inward from the periphery. The most striking evidence of this is the fact that masses of old pigment appear near the periphery as if they had been carried outward by the growth of the new tissue. These abnormal tissues, in the case of hermit crabs and Palæmonetes, lie close against the cuticle and several layers of the cells are flattened as if they were the oldest cells and had been pressed against the cuticle by the multiplication of the cells beneath. In these cases a true hypodermis is not distinguishable. These facts suggest that the migration and pathological development of the old retinulæ are responsible for most if not all of the cases of abnormal pigment deposition. This of course does not explain what induces this pathological development.

The initial cause of this development, in the cases where an abundant network of tissue has developed, was perhaps due to some infection at the time of the operation. This is suggested by the fact that, in the crayfish eye described above, a great deal more tissue had developed abnormally in sixty-two hours than is usually developed normally in ten days or two weeks, and by the fact, also, that in the eye of Palæmonetes viridis a very unusual amount of new tissue had developed during the first nine days after the injury. The more frequent cases in which the pigment secreting cells appear as rounded cells, containing polymorphic nuclei similar to the disintegrating retinular cells, seem to be produced by causes somewhat different. In some specimens examined some time after the injury these cells show no signs of rapid multiplication. It seems probable that these cells are old retinulæ that have retained one of their characteristic functions, the secretion of pigment. Since it is an observed fact that the old retinulæ become metamorphosed and wander to different parts of the stump where they have been found dividing amitotically.

While the above facts are strongly in favor of the conclusion that the abnormal pigment-secreting tissue is due to the development of old retinular cells yet the proof is not absolute. A series of stages of this development, not more than two days apart, would have to be examined in order to be certain of the absolute truth of this tentative conclusion.

2 *Eye Stumps that Show No Regeneration*

It now remains to consider the other phase of the subject outlined in this section; namely, those cases in which there is no regeneration further than the healing of the stump. A number of these cases present anomalies in that there is no apparent reason for their failure to regenerate. There were among the hermit crabs several parallels between those that regenerated an eye and those that did not, so far as conditions were concerned. In a series of fourteen hermit crabs that had the ommatidial portion of the eye removed five regenerated an eye and nine did not. All were kept as nearly as possible under the same conditions. The part of the eye removed in the original operation was about the same for each individual. All were operated upon at the same time in the same way. Some of those that regenerated an eye and some that did not moulted upon the same day after the operation. Consequently the physical condition of these specimens were apparently similar.

Compare Fig. 13 and Fig. 21. Each of the hermit crabs from which these figures were taken moulted twelve days after the operation. The hermit crab from which Fig. 13 was taken was killed at the end of thirty-eight days and the other at the end of sixty-seven days. The latter lived nearly twice as long yet it shows no signs of regeneration. More of the optic ganglion remains in the stump shown in Fig. 21 than in Fig. 13. Again, compare Fig. 15 with Fig. 21. The two crabs from which these figures were taken were operated upon at the same time, moulted approximately upon the same dates and were killed sixty-seven days after the operation. The stump shown in Fig. 21 shows no regeneration while the one shown in Fig. 15 has regenerated an eye perfect in all of its details.

The number of cases might be multiplied but these given are sufficient to show the parallels presented by individual cases. Instances of this sort are confined in great part to hermit crabs. A number of shrimp, however, failed to regenerate even when the optic ganglion was not injured. The same is true for Crangon which in several instances failed to regenerate normally even after the removal of only a small part of the eye.

In most cases sections of such eyes that did not regenerate show no recognizable pathological conditions. In the case shown in Fig. 21, however, there was found what seemed to be the beginning of pathological pigment development. Externally there were no signs of pigment formation. The regenerated tissue consisted of a heavy cuticle, a hypodermis and some loose strands of tissue extending from the hypodermis to the distal end of the stump of the optic ganglion. Grouped at the end of optic ganglion stump and scattered in the loose tissue above it were a few cells of the characteristic pigment-secreting type. But none of these cells had yet become densely filled with pigment (Fig. 75*e*). It seems rather improbable that so little abnormal tissue in which scarcely any secretion of pigment had taken place could have been the sole cause in the prevention of normal regeneration. Particularly is this true when it is remembered that instances have been observed in which practically complete ommatidia were regenerated in eyes containing great masses of abnormal pigment (Figs. 19, 25).

VI REGENERATION AFTER REMOVAL OF THE GREATER PART OR ALL OF THE OPTIC GANGLION

There now remains for discussion those cases in which the whole or most of the eye stalk was removed and consequently either all or the greater part of the optic ganglion. Palæmonetes, Crangon and hermit crabs will each be considered independently since the differences presented by them are such as to require separate treatment.

A HERMIT CRABS

Of a total of sixty hermit crabs operated upon twelve died as a result of the operation, a loss of 20 per cent as against 55 per cent of the Palæmonetes after a similar operation. Thirty-six of these remaining crabs moulted from one to three times and lived from twenty-three to one hundred and ninety-four days. These thirty-six crabs fall into two groups: those that regenerated an antenna-like appendage in place of an eye and those that showed no particular regeneration.

1 Regeneration of Heteromorphic Appendages

Ten crabs in all regenerated an appendage from the old eye stump. In but one case was more than thirty-two days required for the appendage to become apparent. All of these appendages are very small none exceeding in length the normal eye stalk. It is probable, however, that they would have increased both in diameter and length had the experiment covered a longer period of time. None were distinguishable before the occurrence of a moult. In each case recorded the appendage appeared after the first moult. Most of these appendages were definitely segmented after the first moult, in some instances several segments being developed within twenty-one or two days. But none show any indication of being divided into parts corresponding to the exo- and endopodites.

Figs. 23 and 30 show two appendages that were present twenty-one and twenty-two days respectively after the injury. Neither appendage exceeds in length the squame at the base of the normal eye stalk which measures but little more than one-fourth of the whole length of the normal stalk. One appendage bears a considerable number of large tubular hairs. The other shows none whatever. Each is seen to consist of several segments. Five segments are distinctly visible in Fig. 23 while in Fig. 30 there are six or seven though they are not distinctly differentiated. In Fig. 30 the appendage projects outward at a broad angle. Fig. 23 is unique in that it curves in toward the median line and suggests in its general shape and position the squame at the base of the opposite eye. The bifid tip of this appendage is probably due to some injury that occurred at the time of the moult. This explanation is suggested by an examination of the specimen.

Figs. 34 and 45 represent two other appendages that appeared twenty-nine and thirty-two days, respectively, after the injury. These types differ somewhat from the preceding two. It is to be noted in both cases that the original operation did not include the squame at the base of the eye. This is a good indication that at least a part of the proximal segment of the optic ganglion was left. The specimen shown in Fig. 34 was sufficiently transparent so that the optic nerve could be observed extending into the base of the segmented appendage. The specimen from which Fig. 45 was taken was fixed in Flemming and the consequent darkening of the tissues prevented an accurate determination of the length of the stump of the optic nerve. But the nerve stump could be seen extending well into the base of the new appendage.

The appendage shown in Fig. 28 developed in twenty-four days with the intervention of one moult. It is of interest because of the indications that the optic nerve has extended through almost the entire length of the regenerated appendage. It is also of interest because of the ganglionic swelling that appears to be associated with the nerve in its distal half.

Fig. 41 shows an unique type in that the appendage is curved closely back until the free end almost touches the head. Although this appendage is made up of several segments it was rigid from its first appearance.

The remaining examples of these appendages are of approximately the same character as those figured. They belong chiefly to the type shown in Fig. 34, except that two of them show a larger number of tubular hairs. One of these belongs to a specimen that moulted twice and was not killed for sixty-seven days after the operation. The regenerated appendage shows but little advance over those that were fixed at the end of half that time. It is still no longer than the normal eye stalk and shows no greater number of sensory hairs than are seen in Fig. 30. The additional facts obtained from an examination of the sections will be referred to at the close of this section in the general discussion of their significance.

2 *Cases that Show No Especial Regeneration*

As was stated above out of the thirty-six crabs that moulted one or more times only ten developed heteromorphic appendages while twenty-six showed no particular regeneration. The proportion is a little more than 30 per cent to a little less than 70 per cent in favor of those that showed merely a healed over stump.

The stumps that show no actual regeneration present a variety of shapes and characters. None of them, however, show any signs of pathological pigment development. From all appearances the failure to regenerate in most instances was due to a lack of sufficient regenerative activity to produce the new tissue necessary. In some cases where the eye was taken off even with the head the wound healed over leaving a smooth surface, not so much as a slight elevation marking the former position of the eye. In most instances, however, a longer or a shorter stump remained. It is impossible to determine by surface examinations how large the stump was originally for it decreases in size after the operation. Sometimes the stump of the optic nerve and ganglion shrinks to two-thirds of its original volume. Fig. 31 shows a short rounded stump which evidently contains a part of the proximal segment of the optic ganglion. The stump, originally as broad as the base of the opposite eye, has, after one moult twenty-three days after the removal of the eye, shrunk to one-half of the original mass. The remains of the optic nerve seem to come flush against the end of the stump, showing that no new tissue has been developed distal to it. Fig. 39, thirty-two days after the injury, shows a stump more than one-third the length of the normal eye. Yet sections show no indication that any definite structure is being regenerated. It is useless to multiply figures on this phase of the question. They only serve to show how completely is lacking any indication of regeneration.

The following table will serve to show that time cannot be considered the chief factor in regeneration.

No. of specimen	Experiment begun	Closed	Days	Moults	Regeneration
1	October 16	June 30	106	one	none
2	November 27	June 48	158	one	none
3	October 16	April 2	194	one	none
4	May 26	June 16	21	one	segmented appendage
5	May 26	July 4	39	one	none
6	July 9	September 3	56	one	none
7	July 9	August 3	25	one	segmented appendage
8	July 9	August 16	39	one	segmented appendage
9	July 9	September 14	67	one	none
10	July 9	August 6	28	one	segmented appendage

The last five examples given in the preceding table are taken from the same series. Evidently the conditions here were more favorable than usual. The original number of the series was twenty-five. Six of these died either from the effects of the operation or soon afterwards. Of those remaining five others were lost through an accident. Out of the fourteen for which there is a complete record nine developed heteromorphic appendages and all of them within thirty-three days. Two of the remaining five, which moulted at the end of twenty-one days and then died, might perhaps have developed an appendage had they lived through a second moult. Each of them showed a very small bud where the eye had been removed.

B CRANGON

1 Regeneration of Heteromorphic Appendages

In some respects Crangon appears to be a favorable form for experimental work. They are less disastrously affected by the operation than the others worked upon. The entire eye was removed from twenty-two Crangon and not one of the number died from the effects of the operation.

This entire number was of the same series. The experiment covered a period of thirty-three days, August 3 to September 4, inclusive. During that time with one exception each individual moulted at least once and fourteen moulted a second time. Three of those that moulted but once were eaten by their comrades soon after the moult. The evident hardiness of the Crangon and the frequency of the moults would seem to be favorable conditions for regeneration.

Results, however, show only one individual that regenerated a heteromorphic appendage, the others showing no regeneration. Fig. 38*a* and *b* shows surface views of this one regenerated appendage. The animal which developed it moulted on the fourth day after the operation. At that time there was no evidence of regeneration. Seventeen days later another moult occurred and an appendage of six segments, with sensory hairs near the tip, appeared. The appendage measures four-fifths of the length of the eye on the opposite side and projects forward at the same angle. The outline of the optic nerve can be seen extending through the proximal half of the appendage.

2 Cases that Show No Especial Regeneration

Twenty-one out of twenty-two Crangon showed no regeneration. Four of these died within nine days after the operation and so perhaps should not be counted either way. There were then seventeen negative cases against a single positive case.

The eye stalks of Crangon are very short and the sections of the optic ganglion are crowded very close together, and extend well into the base of the stalk. Hence it not infrequently happened that a part of the ganglion remained in the stump. A number of these stumps have been sectioned and none of them show any regenerated tissue except the hypodermis and cuticle. Spots of pigment are often seen at the end of the stump but since the whole stalk of the normal eye is heavily pigmented this does not seem to be significant. Figs. 24, 37, 40 and 42 show a variety of appearances which the stumps presented. The accompanying table shows the number of moults which occurred.

No. of specimen	Experiment begun	First moult	Second moult	Third moult	Date of death	Regeneration
8	August 3	August 9	August 21	none	September 2	none
10	August 3	August 10	August 22	none	September 4	none
13	August 3	August 12	August 28	none	August 28	none
15	August 3	August 4	August 13	August 19	September 2	none
17	August 3	August 14	August 29	none	September 4	none

In some cases the eye stump is extremely short while in others it is longer so that a part of the ganglion remains. All of the specimens included in the table except No. 17 have been sectioned but none of them show any signs of regeneration. Sections of No. 10 show that nearly half of the optic ganglion was left but no regeneration is taking place. A very much folded and wrinkled cuticle with short hairs projecting from it covers the stump. Even No. 8 (Fig. 37), short as it appears, is found to contain the proximal end of the optic ganglion. In this case the stump has merely healed over but no new tissue has developed. In several other instances not shown in figures the eye had been totally removed so that not even a short stump is visible. In most such cases the cuticle is wrinkled over the spot where the eye had been. The wrinkles and folds on some of the stumps figured shows the common tendency. These folds are chiefly due probably to the shrinking of the inner tissues of the stump.

C PALAEMONETES

Out of nearly three hundred Palæmonetes not a single individual regenerated any sort of an appendage when all or nearly all of the optic stalk was removed. It is true that more than 50 per cent of them died from the operation or soon after. Often half or two-thirds of a series died within twenty-five or thirty minutes after the operation, and in some instances the proportion was still greater. (See Table 1.) Palæmonetes were by far the least resistant of any of the forms operated upon. There were, however, over sixty individuals that lived from twenty to one hundred and twenty-four days and moulted from one to three times.

Considering the results of these experiments it may be said that Palæmonetes vulgaris does not regenerate an antenna-like appendage in place of an eye. Herbst would, perhaps, insist that these results were due to a lack of time or to a failure to remove all of the optic ganglion. This latter objection in many cases could not be urged. The eye stalk in Palæmonetes is long and the optic nerve extends well into its base. And in these experiments the eye was so completely removed that not even the vestige of a stump remained. Consequently there was no possibility of leaving any part of the optic ganglion. Part of the brain even was removed with the eye in two series. In regard to the other objection naturally there is no positive proof that results might not have been different in a longer period of time. There are strong reasons, however, for believing that time would have made no essential difference. Chief among these reasons is the fact that in the regeneration of any other organ Palæmonetes needs but little more time than the hermit crabs and less time than Crangon. In three parallel series of experiments upon the regeneration of the first antenna after its total extirpation it was found that Palæmonetes regenerates a first antenna as quickly and as perfectly as either Crangon or hermit crabs. In another parallel series of experiments upon the regeneration of the second antenna it was found that Palæmonetes regenerates this appendage rather more rapidly than either hermit crabs or Crangon. Palæmonetes may regenerate a first or second antenna in about thirty days. Neither hermit crabs nor Crangon regenerate these appendages in less time. In the regeneration of a functional eye it was seen in a previous section that hermit crabs regenerate rather more rapidly than Palæmonetes but that Palæmonetes regenerate more rapidly than Crangon. Palæmonetes may regenerate a functional eye in thirty to thirty-five days. It is seen, therefore, that appendages are regenerated by Palæmonetes in approximately the same time as they are regenerated in hermit crabs and Crangon. Consequently it does not seem to be assuming too much to express the conviction that a greater amount of time would have made no essential difference in the results of these experiments in which the entire eye of Palæmonetes was removed.

Below is a brief table showing results obtained by removing the entire eye of Palæmonetes. This table does not include all the individuals of any one series but it is entirely representative.

No. of specimen	Experiment begun	First moult	Second moult	Experiment closed	Days	Regeneration
1	November 5	November 27	December 30	February 3	90	none
2	November 9	November	January	March 13	124	none
3	November 9	November	January	February 12	95	none
4	January 1	January 15	February 7	February 24	55	none
5	January 1	February 20	none	February 28	59	none
6	March 5	April 24	none	April 24	50	none
7	April 19	May 5	June 8 & 25	July 3	71	none
8	May 10	June	none	July 4	55	none
9	July 10	July 26	August 6	September 2	54	none
10	July 10	July 27	August 9 September 6	September 14	66	none
11	July 20	July 30	none	August 18	29	none
12	July 10	July 18	July 31	August 3	24	none
13	July 20	July 30	August 14	August 15	27	none
14	July 20	July 30	August 10	August 11	22	none
15	July 20	July 24	none	August 20	30	none
16	July 30	August 8	August 27	September 4	36	none

It will be seen that Palæmonetes have been under observation practically every month in the year. The results in each instance are negative. Fig. 35*a*, *b*, *c* represent some of the stumps that show new tissue distal to the nerve stump. Most of the cases, however, regardless of the time of the experiment and the size of the stump, are similar to the one shown in Fig. 33, No. 13 in the table. Fig. 33 shows the nerve stump flush against the healed end. The indications from sections and surface views are not such as to lead one to expect that further regeneration would have ever taken place. Fig. 26 represents the only stump that even suggests the development of a heteromorphic structure. As the table shows this specimen lived only twenty-four days, during which time it moulted twice, and regenerated the tiny mass of new tissue represented by the darkly stippled portion of the figure. The eye was completely removed, the cut coming at the level of the attachment of the eye to the head, represented in Fig. 26 by the line *a–b*. Fig. 27 represents a more highly magnified view of the stump.

The remaining figures in the series, Fig. 35*a*, *b*, *c*, show the maximum regeneration, yet in none of these cases did the experiment cover more than thirty days. Apparently regeneration in most cases proceeds to the forming of the hypodermis and cuticle, which may be extended slightly beyond the nerve trunk by loose strands of connective tissue, and then stops. Fig. 35*c* shows more than the usual amount of new tissue. The line *a–b* represents the level of the union of the eye with the head. The unshaded central part of the eye stump shows the remains of the optic nerve; the shaded peripheral portion shows the new tissue. Neither sections nor surface examinations give the slightest evidence of the regeneration of nerve fibers, or of any special differentiation of the regenerated tissue.

D THE HISTOLOGY OF THE HETEROMORPHIC APPENDAGES

The microscopic structure of the antenna-like appendages has not been considered in great detail because suitable material has been wanting. In the whole series of experiments only ten hermit crabs and one Crangon ever regenerated a heteromorphic appendage in place of the excised eye. Several of these died and were preserved in alcohol. From such material no detailed results were obtainable. Again, the only sections of any particular interest and value are longitudinal ones. These heteromorphic appendages were so small and so curved that it was almost impossible to obtain satisfactory longitudinal sections.

A few points of interest, however, have been observed. These for the most part serve to corroborate the observations of Herbst rather than to add to them. An examination of the appendages in toto show that the old optic nerve either extended as a nerve trunk through the greater part of the length of the regenerated appendage or that other structures were developed in the new appendage which appeared to be continuous with the old optic stump (Figs. 30, 34, 38). Sections confirm the observations made from surface examinations. A large number of intermediate stages would be necessary, however, to determine whether the regeneration of the nerve trunk had been from the optic nerve stump outward or whether peripheral regeneration had developed nerve fibers inward which unite with the optic nerve stump. The fact, however, that the nerve trunk appears more distinctly differentiated in the proximal part of the appendage than in the distal may probably be regarded as an indication that the regeneration proceeds from the proximal end outward.

Sections of these appendages show that the interior is chiefly occupied by nerve cells and fibers. The nerve fibers appear to be continuous with the nerve fibers of the old optic nerve stump. The nerve cells are grouped into ganglion-like masses which are scattered pretty generally through the length of the appendage. The brain sheath is continuous with the loose fibrous sheath which envelops the mass of nerve cells and fibers.

Fig. 77 shows a somewhat diagrammatic section through the brain and the proximal end of a heteromorphic appendage that developed within sixty-seven days after the operation. It was necessary to combine two sections in order to show the continuity of the optic nerve with the nerve trunk of the appendage. There can be no doubt, however, that they form a continuous structure. One feature is noticeable both in sections and in whole preparations. That is, that the optic trunk leading to the regenerated appendage is much smaller in diameter than the one opposite. This fact suggests that probably only a part of the fibers of the optic nerve have persisted (Figs. 34, 38).

Large blood sinuses are present in the heteromorphic appendages. Aside from these no tissues are apparent except the hypodermis and the fibrous sheath which encloses the nerve bundles and the nerves themselves. In some instances muscles are found in the base of the appendage but these are probably remains of the base of the eye stump.

Material has been insufficient to make detailed observations upon the character of the masses of sensory cells found in the ganglion-like groups throughout the appendage. Sufficient observations have been made, however, to warrant the conclusion that they are concerned with the innervation of the hollow sensory hairs. In a few instances processes have been traced into the bases of the hairs which open by a wide mouth into the interior of the appendage (Figs. 78 and 79).

Herbst has considered the microscopic structure of these heteromorphic appendages in considerable detail and has examined a number of different stages. He describes the nerve cells as grouped into spindle-shaped ganglia with groups of nerve fibers extending from each end of the spindle-shaped masses, the distal bundle of strands being connected with the sensory hairs while the proximal bundle passes inward toward the brain. None of the stages examined by Herbst were younger than about six months, however, and consequently any structures that had developed would likely be much more definitely organized than in the appendages examined in this series of experiments.

Herbst considers that these ganglion-like groups of cells have developed from the hypodermis and that in the earlier stages they have no direct connection with the brain. In later stages, however, he describes the proximal bundles of the several ganglia as uniting to pass inward to the brain. But in most cases at least he considers that there is no union with the old optic nerve and consequently that the connection of the appendage with the brain is secondary. He mentions the similarity between these epithelial sense cells and those found in the first antenna, homologizing the sensory hairs which are found on the appendage with the olfactory setæ found upon the first antenna. Finally he comes to the conclusion that both in form and structure the heteromorphic appendage shows that it should be regarded as a rudimentary first antenna. The structure of the heteromorphic appendage regenerated by the hermit crab agrees in certain respects with the observations of Herbst upon the structure of the heteromorphic appendages regenerated by other forms. In other respects the observations made upon hermit crabs are not sufficiently extensive to have any particular weight either way. The most significant difference, however, between these observations and Herbst's is in regard to the relation of the old optic nerve stump to the nerve bundles extending through the appendage. In the heteromorphic appendages regenerated by the hermit crabs there are several cases in which there can be no doubt as to the continuity of the optic nerve with the nerves in the appendage (Figs. 28, 34, 38). Further, these are found in stages younger than any spoken of by Herbst.

The continuity of the optic nerve stump and the nerve trunk of the heteromorphic appendage will be considered in all of its aspects in the general consideration of the problem of such heteromorphic regeneration.

E GENERAL CONSIDERATION OF REGENERATION FOLLOWING REMOVAL OF ENTIRE EYE

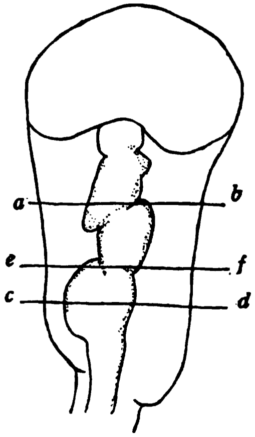
It has been seen in some cases that hermit crabs and Crangon regenerate an antenna-like appendage in place of an eye. On the other hand, the species of Palæmonetes used in this series of experiments has never shown any indication of such regeneration. In view of this, the question which naturally arises is why do we not find antenna-like appendages growing from the eye stumps of Palæmonetes vulgaris, when hermit crabs and Crangon kept under the same condition do regenerate these structures, and when the phenomenon is of pretty general occurrence among the Decapods. Herbst has observed the development of an antenna-like appendage from the eye stumps of a number of stalked-eyed Crustacea belonging to different families. He has even secured a few cases of this heteromorphosis in another species of Palæmonetes (P. varians). Morgan ('99) was the first to make the observation for hermit crabs and a like phenomenon has been noted for three species of crayfish, Cambarus virilis and C. gracilis (Steele '04) and the blind crayfish, C. Pellucidus testii (Zeleny '06).

Widespread as the phenomenon appears to be, however, no satisfactory explanation of the cause of such heteromorphic regeneration has yet been suggested. Also an explanation of the negative cases, that is, where no particular regeneration takes place, is equally wanting. In the explanation of any phenomenon it is essential that negative cases be taken into account before any general conclusions are drawn. As has been pointed out above, even among the hermit crabs where the heteromorphic appendages appeared most frequently, in by far the majority of cases no regeneration took place. There was in these experiments a single series of hermit crabs in which nine out of fourteen individuals regenerated a heteromorphic appendage. In the light of this, we should perhaps be safe in concluding that for hermit crabs failure to regenerate may often be due to external conditions. But this would still explain nothing for Crangon and Palæmonetes.

All of the Crangon experimented upon belonged to the same series and were kept as nearly as possible under precisely the same conditions. Yet but one out of the original twenty-two developed an antenna-like appendage notwithstanding there were fourteen others that lived as long or longer and moulted as frequently. In so far as it was possible to determine the question, the physiological activity of the fourteen that showed no regeneration was equal to that of the one individual that did regenerate the appendage.

Extensive series of Palæmonetes were operated upon at the same time with the hermit crabs, and were kept under similar conditions. Yet, as has been seen not one regenerated the antenna-like appendage. From this it appears evident that, whatever variations in results may be accounted for by differences in external conditions, the primary answer to the question must be sought elsewhere.

It may be objected that in operating upon the eye the entire optic ganglion was not always removed. This, however, could not be offered as an objection in every case. In all three of the forms there were many instances in which not a vestige of the optic ganglion remained, and yet no regeneration resulted. Besides there is evidence that in some cases hermit crabs regenerate an antenna-like appendage when part of the ganglion has been left in the eye stalk. We have then the following conditions for hermit crabs at least. First, when the cut comes at a level which leaves as much as two sections of the optic ganglion intact an eye may regenerate (*a–b*, text Fig. 2). Second, when the cut is made at the base or slightly above the base of the eye stalk (*c–d*, text Fig. 2) so that little or none of the optic ganglion remains the regeneration of an antenna-like appendage is possible. Lastly, if the eye is removed at a level intermediate between *a–b* and *c–d* (text Fig. 2) no regeneration follows.



Text Fig. 2 The line *a–b* represents approximately the level from which a hermit crab may regenerate an eye. From the level of the line *c–d* or below it a heteromorphic appendage may regenerate. No regeneration takes place from intermediate level, *e.g.*, from the level *e–f*.

It is possible, perhaps even probable, that the character of the hypodermis differs more or less at these different levels. It is even conceivable that the hypodermis should be capable of one sort of regeneration at the level *a–b* or above it, and of another sort at the level *c–d* or below it; but there is certainly no apparent reason why no regeneration whatever should take place if the eye is removed at a plane intermediate between these two levels. So far as careful microscopic examination can determine there is no difference in the hypodermal cells underlying the cuticle proximal to the basement membrane. Whatever differences in character may exist between the hypodermal cells over different regions of the eye, the results of this whole series of experiments suggest the inference that presence or absence of a maximum amount of the optic ganglion is a controlling factor in determining the character of the regeneration. The fact that no regeneration takes place from levels intermediate between *a–b* and *c–d* is in itself evidence that internal conditions are different at these intermediate levels than from a higher or a lower level. So far as the optic ganglion may be a controlling factor the difference in conditions may be due either to a difference in the character of the ganglion cells or to the reduced ganglionic mass. From the structure of the optic ganglion (Parker '90 and Kenyon '97) it is probable that not until the lower level *c–d* has been reached have the peripheral terminations of the optic nerve fibers been seriously interfered with. Both Parker and Kenyon mention the fact that a part of the optic nerve fibers have their cellular origins located in the brain. The fact that this heteromorphic appendage never regenerates except from this lower level suggests that there may be a causal connection between the regeneration of the heteromorphic appendage and the destruction of the distal terminations of the optic nerve fibers. With their peripheral terminations destroyed there might probably be a tendency on the part of the optic nerve fibers to grow outward and form new terminations. Since their natural terminations, the cells of the optic ganglion have been destroyed it seems probable that the fibers of the optic nerve stump would behave like those in a nerve stump of an ordinary appendage, *e.g.*, a leg or antenna. This in itself might have a tendency to induce any new tissue that regenerated to differentiate into the form of some sort of appendage.

That this heteromorphic appendage should be antenna-like in form seems probable for two reasons. First, it is the natural tendency of all Arthropod structures to divide into segments. Second, the simplest form of joint found in any appendage is in the antenna. Further, this appendage, although antenna-like, shows a much greater variety in form than any ordinary regenerated appendage and the joints formed are often irregular and incomplete. This fact suggests that the regeneration was not influenced by a fixed set of internal conditions. In the usual cases of regeneration and embryonic development, whatever the determining factor or factors may be, it is recognized that we may expect certain structures to appear in connection with a given set of external and internal conditions.

In the development of this heteromorphic appendage, however, conditions seem more variable. As a consequence it shows considerable variety of form. In some cases the appendage is but little more than a slender horn-like projection, in other cases the appendage may be curved inward toward the median line, project forward at the angle of the eye or curve backward until the free end touches the margin of the head. (Compare Figs. 23, 38 and 41.) Again from the very first moult the appendage may appear as a single flagellum-like structure or as a pair. None of the hermit crabs, however, have regenerated a heteromorphic appendage composed of two flagellum-like parts. But in my previous observations upon crayfish (*loc. cit.*) two or three instances were noted in which the appendage appeared double at the time of the first moult. Herbst has also noted what he regards as an endopodite and exopodite in several instances. The appearance of the single structure in some cases and the double one in some others can perhaps be explained by the supposition that the nerve fibers become separated into two masses in some instances and remain as a single trunk in others. Miss Reed (*loc. cit.*) found that when the stump of the leg of a crayfish or hermit crab was split longitudinally in some instances two legs were regenerated from a single stump and in other cases only one. Sections of such legs showed that the end of the nerve stump had been split in the cases in which two legs regenerated and that the nerve stump had not been split when only one leg was regenerated. A similar result might follow in the development of the heteromorphic appendage if the nerve trunk became separated into two bundles by the interposition of another sort of tissue.

An explanation of the antenna-like form of the heteromorphic appendage having been suggested, attention should now be directed toward an explanation of its inner structure, which is also found to be antenna-like. That its inner structure should be antenna-like might be expected since its innervation is associated with a region of the central nervous system that is particularly concerned with the innervation of the special sense organs, and since its outward form is antenna-like it is rather to be expected that the inner structure would also conform more or less to the antenna type.

It seems evident that the ganglionic groups of sense cells which are found in the heteromorphic appendage, belong to the general peripheral nervous system found so widely distributed among the different Arthropods. The groups of cells and the associated sensory hairs are equivalent to the "Hautsinnesorgane" of vom Rath ('94). Ost (*loc. cit.*), however, does not regard these sense cells as true ganglion cells, as Herbst does. In the regenerating antenna of Oniscus, Ost finds the nerve fibers regenerating from the central stump and the groups of sense cells differentiating from the hypodermis. The regenerating nerve fibers come from the end of the nerve stump, extend to the periphery and intermingle with the sense cells. Bethe ('96) considers that the peripheral nervous system of Arthropods differs both in function and origin from the central nervous system. Holmgren ('95) regards it as a sort of sympathetic system.

That cutting the peripheral terminations of the optic nerve may induce the regeneration of a heteromorphic appendage seems to receive some support from the results obtained by Zeleny upon the blind crayfish. Although reduced in size the optic ganglion is still present in the rudimentary eyes of blind crayfish. On the other hand the ommatidial structures are entirely wanting. So long as the vestigial eye remains undisturbed there seems to be no tendency toward the development of an antenna-like organ. But when the optic ganglion is removed a heteromorphic appendage appears. Such appendages are apparently functional as sense organs and Zeleny concludes that in the blind crayfish a non-functional organ has been replaced by a functional one.

The suggested explanation for the outgrowth of the heteromorphic appendage also carries with it an implied explanation of the non-appearance of a heteromorphic structure in place of a somatic appendage. The nerve trunk of an appendage is associated with ganglion cells only at its central end, not with ganglion cells at its peripheral end, as distinguished from the optic nerve in its relation to the optic ganglion, consequently in removing an appendage no parts have been removed that would not be likely to again regenerate in a similar manner. While in animals as highly specialized as the hermit crabs we do not find the ganglion parts of the nervous system regenerating.

For the negative cases that appear after the entire optic ganglion has been removed, it is evident that no real explanation can be offered until a more adequate understanding of the process of growth and development has been reached. Although we may fully recognize the fact that great differences exist in the physiological activity of the various individuals and that the external conditions are subject to numerous variations, these facts alone will not account for the great number of negative cases which result. In addition to these it seems necessary to recognize an individual variation in the quality of the tissues. Nothing short of some specific inherent individual difference seems sufficient account for the fact that only an occasional hermit crab regenerates a heteromorphic appendage. The ability to regenerate a heteromorphic appendage in place of an eye which appears as an individual variation in hermit crabs and Crangon and other genera seems to be entirely wanting in at least one species of Palæmonetes. Or if not entirely wanting it appears so rarely that even after a great number of experiments and observations it is apparently absent.

In summing up the foregoing discussion it is apparent that a weight of responsibility has been placed upon the nervous system. Numerous observations, however, have left no doubt that the nervous system does exercise an important physiological influence upon the other tissues of the body, both in ordinary growth phenomena and in regeneration. Child ('04) observed in operating upon Leptoplana that if more than half of the cerebral ganglion was removed a new head did not regenerate. This was true regardless of the plane in which the cut was made, a fact which seems to indicate that the mass of nervous material is an important factor in the case of Leptoplana at least. Wilson ('03) discovered that after the larger chela of Alpheus had been removed, cutting the nerve in the smaller one prevented it from growing into the form of the larger one when, however, the large chela had been removed and the nerve in the small one left intact, the small chela developed into the form of the large one. It has been noted above that Miss Reed found she could obtain the regeneration of the double chelæ. The experiments of Schaper ('98), Harrison ('03), Barfurth ('01), Goldstein ('04) and others have shown that the early stages of embryonic development and of regeneration are apparently independent of the nervous system. But the same experiments have also shown that the later stages of growth and differentiation are very largely influenced by the part of the nervous system which normally innervates the regenerating or developing parts; other instances might be mentioned but a sufficient number have been given to convince one that in very many instances there is an important connection between the part of the nervous system immediately concerned and the regeneration of the other tissues and structures.

VII REGENERATION AFTER SPLITTING THE EYE LONGITUDINALLY

Several series of Palæmonetes were operated upon by having the eye split longitudinally (Table 1). Although in the regeneration of any part of the eye the new tissue is derived from the hypodermis the results obtained from the experiment of splitting the eye seem to indicate that injury to the optic ganglion is of great importance. In many cases at least splitting the eye could not have resulted in serious injury to the hypodermis yet in no case did regeneration follow if the optic ganglion had been injured. Whether or not regeneration followed the operation apparently depended upon the depth of the split. If the split extended through the ommatidial portion only and the optic ganglion remained uninjured, the ommatidial portion degenerated and new ommatidia were in some cases regenerated. On the other hand, if the split extended into the optic ganglion the whole ommatidial portion and the whole or part of the optic ganglion degenerated.

In some cases not even a vestige of the eye remained; in others, stumps of considerable length persisted. But in no cases where the split extended into the optic ganglion was there any sign of regeneration.

Figs. 16 and 22 represent instances in one of which an eye regenerated and in the other there were no signs of a regenerating eye. The specimen from which Fig. 16 was taken lived sixty-five days after the eye was split. The regenerated eye is about six-sevenths of the length of the normal eye. Sections show that new ommatidia have regenerated. The eye is not altogether normal in structure, however. The eye stump shown in Fig. 22 was taken from an individual that lived seventeen days after the operation. Apparently the entire optic ganglion has degenerated. There are no definite indications of regeneration. The stump of the optic nerve tapers to a point, perhaps indicating that degeneration is still incomplete. The stump is little more than one-third the length of normal eye.

No additional facts of importance were gained from the experiment of splitting the eye. These results obtained serve chiefly as additional proof that an injury to any part of the eye is followed by widespread degeneration of the tissues and that in the case of Palæmonetes, after an injury to the optic ganglion usually no regeneration takes place.

SUMMARY

In summing up the results of the experiments discussed in this paper the following points are to be noted:

1 The death of the animal which so frequently follows immediately upon the operation is perhaps due rather to its effect upon the nervous system than to loss of blood.

2 The healing of the wound takes place by the formation of a provisional crust over the cut surface and later by the development of a new cuticle beneath this crust.

a The crust is formed of hypodermal cells and a chitinous secretion. Intermingled with this are blood cells and the cells of the injured tissues. From two to three days are required for the formation of the crust.

b The new cuticle is secreted before a continuous hypodermis has formed over the wound. It is continuous with the inner layers of the cuticle over the eye stump.

3 New hypodermal cells over the ommatidial region may arise in two ways; either by the transformation *in situ* of corneal hypodermal cells into less specialized, actively multiplying hypodermal cells, or by the proliferation of new hypodermal cells inward from the edges of the cut.

4 Any injury to the eye is always accompanied by extensive degeneration of the remaining tissues. Sometimes the entire eye suffers destruction.

5 The rate of regeneration is considerably affected by the rate of disintegration and the removal of injured parts.

6 Active regeneration may be in progress at the periphery while deeper below the surface the injured structures are not yet removed.

7 In the regeneration of an eye all of the new structures arise from the hypodermis.

8 Multiplication of cells takes places by amitotic divisions.

9 The cells for the retinulæ are the first to differentiate from the hypodermis. Their differentiation may begin before a continuous hypodermis has developed.

10 The retinular nuclei move inward from the periphery, elongate and divide along their radial axes, and extend proximal processes through the basement membranes to the optic ganglion. Thereby nervous connections are established in the regenerating region.

11 Not until after the retinular processes have extended into the optic ganglion is the differentiation of cones established. The cones differentiate from the periphery inward.

12 The rhabdom is developed from the inner ends of the retinular cells and is at first present as a slender homogeneous rod of uniform diameter, which extends from the inner end of the cones to the basement membrane. The spindle shaped enlargement of the rhabdom does not appear until after all the other parts of the ommatidium have been differentiated.

13 The hypodermis does not become a true corneal hypodermis

and secrete corneal facets until after all of the other ommatidial structures have been differentiated. Corneal facets are never apparent until after more than one moult has taken place.

14 Ommatidia do not differentiate at a uniform rate in all parts of the regenerating eye.

15 In Palæmonetes regeneration of perfect ommatidia does not take place if the optic ganglion has been injured. Hermit crabs may regenerate a perfect eye after removal of as much as half the optic ganglion. Crangon regenerates an eye very slowly, even when the optic ganglion is uninjured, but there are evidences that ommatidia may differentiate after a part of the optic ganglion has been removed.

16 The rate of regeneration is quite variable in all the species experimented upon, but both hermit crabs and Palæmonetes however may regenerate ommatidia within thirty-five to forty-five days.

17 Splitting the eye of Palæmonetes is not followed by regeneration if the split extends into the optic ganglion.

18 In the breaking down of the injured ommatidia the pigment secreting cells become widely scattered, and the old pigment persists for a long time. Frequent cases of abnormal development of pigment also occur. There are evidences which indicate that this abnormality is due to the pathological development of the broken down retinulæ.

19 After removal of all or nearly all of the optic ganglion, hermit crabs may regenerate a heteromorphic appendage in place of the excised eye. There is, however, apparently a level from which neither an eye nor an antenna-like appendage will regenerate.

20 The nerve-trunk of the heteromorphic appendage forms a continuous structure with the stump of the optic nerve.

21 Removal of the entire eye of Crangon may also be followed by the regeneration of an antenna-like appendage.

22 In no case was there evidence that Palæmonetes vulgaris possessed the ability to regenerate a heteromorphic appendage after the removal of the entire eye.

23 The results of this entire series of experiments points to the

following conclusion. The regeneration which takes place from any level is largely influenced by the presence or absence of the whole or a part of the optic ganglion.

BIBLIOGRAPHY

BARFURTH, D., '01—Ist die Regeneration vom Nervensystem abhängig? Verh. d. Anat. Ges., 1901 (p. 197–201).

BETHE, ALBRECHT, '96—Ein Beitrag zur Kenntnis des peripheren Nervensystems von Astacus fluviatalis. Anat. Anz., 1896, Bd. xii (p. 31–34).

CHILD, C. M., '04—Amitosis in Moniezia. Anat. Anz., Bd. xxv (p. 545–558).

'07—Amitosis as a Factor in Normal and Regulatory Growth. Anat. Anz., Bd. xxx, nos. 11 and 12.

GOLDSTEIN, K., '04—Kritische und experimentelle Beiträge zur Frage nach dem Einfluss des Centralnervensystem auf die embryonale Entwickelung und die Regeneration. Arch. f. Entw.-Mech., Bd. xviii, H. 1, 1904.

'04—Die Abhängigkeit der Muskulatur vom Centralnervensystem während der Embryonalzeit. Eine Erwiderung an Herrn Prof. Neumann. Arch. f. Entw.-Mech., Bd. xviii, H. 4.

GRENACHER, H. '74—Zur Morphologie und Physiologie des facettirten Arthropodenauges. Götten Nachrichten (p. 645–656).

HARRISON, ROSS G., '03—On the Differentiation of Muscular Tissue when Removed from the Influence of the Nervous System. Am. Jour. of Anat., vol. 2, no. 2.

HERBST, C., '96—Ueber die Regeneration von antennenähnlichen Organen an Stelle von Augen. I. Arch. f. Entw.-Mech., Bd. ii, H. 4.

'96—Ueber die Regeneration von antennenähnlichen Organen an Stelle von Augen. II. Versuche mit Sicyonia Sculpta. Vierteljahrsschr. Naturf. Gesellsch. Zürich. Jahrg., 41 (p. 435).

'00—Ueber die Regeneration von antennenähnlichen Organen an Stelle von Augen. III. Weitere Versuche mit total Exstirpirten Augen. IV. Versuche mit theilweise abgeschnittenen Augen. Arch. f. Entw.-Mech., Bd. ix, H. 2 (p. 215–293).

HERRICK, F. H., '89—The Development of the Compound Eye of Alpheus. Zool. Anz., xii, no. 303 (p. 164–169).

HOLMGREN, E., '95—Zur Kenntnis des Hautnervensystems der Arthropoden. Anat. Anz., Bd. xii, no. 19 (p. 449–457).

KENYON, F. C., '96—The Brain of the Bee. Jr. of Comp. Neur., vol. vi.

'97—The Optic Lobe of the Bee's Brain in the Light of Recent Neurological Methods. Am. Nat., xxxi (p. 369–376).

KINGSLEY, J. S., '87—The Development of the Compound Eye of Crangon. Jour. Morph., i, no. 1 (p. 49–66).

McGREGOR, J. H., '99—The Spermatogenesis of Amphiuma. Jour. of Morph. Sup. to vol. xv.

MEVES, 91—Amitotic Division of Spermatagonia of Salamandra. Anat. Anz., Bd. vi.

OST, J., '06—Zur Kenntnis der Regeneration der Extremitäten bei den Arthropoden. Arch. f. Entw.-Mech., Bd. xxii, H. 3 (p. 289).

PARKER, G. H., '90—Histology and Development of the Eye in the Lobster. Bull. Mus. Comp. Zoöl., Harvard, vol. xx, no. 1 (p. 1–60).

'91—The Compound Eyes in Crustaceans. Bull. Mus. Comp. Zoöl., Harvard, xxi, no. 2 (p. 45–140).

'95—The Retina and Optic Ganglion in Decapods, Especially in Astacus. Mitth. Zool. Stat. Neapel., vol. xii, H. 1 (p. 1–73).

'97—Photomechanical Changes in the Retinal Pigment Cells of Palæmonetes and their Relation to the Central Nervous System. Bull. Mus. Comp. Zoöl., Harvard, xxx (p. 275–300).

PATTEN, WILLIAM, '86—Eyes of Molluscs and Arthropods. Mitth. Zool. Stat. Neapel, vol. vi (p. 542–756).

'87—Studies on the Eyes of Arthropods. I. Development of the Eyes of Vespa. Jr. Morph., vol. i (p. 193–226).

PHILLIPS, E. F., '05—Structure and Development of the Compound Eye of the Honey Bee. Proc. Acad. Nat. Sci., Philadelphia (p. 123–157).

RATH, O. VOM, '87—Zur Kenntnis der Hautsinnesorgane und des sensiblem Nervensystems bei den Arthropoden. Zeit. f. Wiss. Zool., Bd. 41, H. 3 (p. 499–589).

REED, MARGARET A., '04—The Regeneration of the First Leg of the Crayfish. Arch. f. Entw.-Mech., vol. xviii, H. 3 (p. 307–316).

ROSENSTADT, B., '96—Beiträge zur Kenntnis des Baues der zusammengesetzten Augen bei den Dekapoden. Arch. Mikr. Anat., Bd. xlvii (p. 748–770).

SCHAPER, A., '98—Experimentelle Studien an Amphibienlarven, Erste Mitteilung. Haben künstlich angelegete Defekte des Centralnervensystems oder die vollständige Elimination desselben einen nachweisbaren Einfluss auf die Entwickelung des Gesammtorganismus junger Froschlarven? Arch. f. Entw.-Mech., Bd. vi (p. 157–197).

'98—Experimental Studies on the Influence of the Central Nervous System upon the Development of the Embryo. Jr. of the Boston Soc. Med. Sci., January, 1898.

STEELE, MARY I., '04—Regeneration of Crayfish Appendages. Univ. of Mo. Studies, vol. ii, no. 4.

WATASE, S., '89—On the Structure and Development of the Eyes of the Limulus. Johns Hopkins Univ. Circ., viii, no. 79 (p. 34–37).

'90—On the Morphology of the Compound Eyes of Arthropods. Studies Biol. Lab., Johns Hopkins Univ., iv. no. 6 (p. 287–334).

WILSON, E. B., '03—Notes on the Reversal of Asymmetry in the Regeneration of the Chelæ in Alpheus Heterochelis. Biol. Bull., vol. 4 (p. 187–214).

ZELENY, C., '05—Compensatory Regulation. Jr. Exp. Zoöl., vol. ii, no. 1 (p. 1–102).

'06—Regeneration of an Antenna-like Organ in Place of the Vestigial Eye of the Blind Crayfish, C. Pellucidus Testii. Sci., vol. xxiii (p. 527).

EXPLANATION OF PLATES

Outlines of all figures were drawn with the aid of a camera. In all of the detailed figures the nuclei were also drawn in with the camera. The magnification is given with the explanation of each figure. As far as possible the figures illustrating the different phases of the subject are numbered according to the number of days the experiment covered.

Reference letters used

a.	anterior.
a.cp	anterior border of carapace.
a.p.c.	abnormal pigment cells.
bm.	basement membrane.
br.	brain.
br.sh.	brain sheath.
c.c.	crystalline cones.
c.hy.	corneal hypodermis.
c.p.	coagulated plasma.
cr.	crust.
ct.	cuticle.
e.s.	eye stump.
cf.	corneal facet.
gl.	ganglion.
het.	heteromorphic appendage.
hy.	hypodermis.
hy.tr.	transformed hypodermis.
m.	muscle.
n.c.t.	new cuticle.
n.e.	normal eye.
n.	nucleus.
n.tr.	nerve trunk.
o.ct.	old cuticle.
c.m.	old muscle.
op.n.	optic nerve.
o.sp.	optic squame.
p.	posterior.
pt.	pigment.
pt.cs.	pigment cysts.
ret.	retinulæ.
ret.n.	retinular nuclei.
rh.	rhabdom.
r.t.	regenerated tissue.
seg.	segments.
s.ct.	sub cuticle.
sn.	sensory hairs.

PLATE I

Fig. 1 Palæmonetes, seven days. One moult seven days after operation. *a*, Dorsal view, and *b*, ventral view. Most of ommatidia removed from ventral side. Pigmented portion appears disorganized. Injured eye measures about four-fifths length of normal eye. × 45.

Fig. 2 Young Palæmonetes, seven days. One moult seven days after operation. Dorsal view. Eye operated upon by thrusting needle into top of ommatidial portion. Nearly half of the ommatidia destroyed. Injured eye measures about three-fourths length of normal eye. × 45.

Fig. 3 Palæmonetes, ten days. One moult seven days after operation. Ventral view. Part of ventral ommatidial portion removed. Pigment irregularly scattered throughout ommatidial region. Very few uninjured ommatidia remains. × 45.

Fig. 4 Palæmonetes eye, nineteen days. One moult eighteen days after the operation. Nearly whole ommatidial portion was removed. Pigment patches remains of old ommatidia. New tissues can be seen arranged in strands on interior edge. × 45.

Fig. 5 Hermit crab, twenty-five days. Regenerated eye, one moult twenty-four days after operation. At least one section of optic ganglion removed. Regenerated eye five-eighths length of normal eye. × 45.

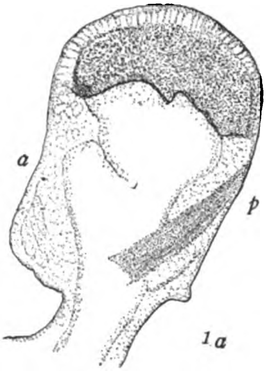
Fig. 6 Palæmonetes, thirty days. First moult seven days after operation; second moult twenty-one days later. Ventral view. Whole ommatidial region destroyed. Upper part of regenerated tissue perfectly transparent. Irregular patches of old pigment remains seen in lower part of ommatidial region. Regenerating eye three-fourths length of normal eye. × 45.

Fig. 7 Crangon, thirty-two days. First moult eighteen days after operation; second moult fourteen days later. Dorsal view. Operation removed upper ommatidial surface. Remains of old pigment apparent. Interior of eye shrunk away from cuticle. Injured eye four-fifths length of normal eye. × 60.

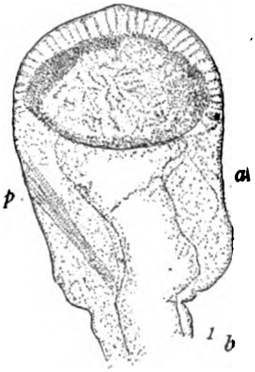
REGENERATION IN COMPOUND EYES OF CRUSTACEA

PLATE I

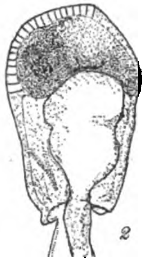
MARY ISABELLE STEELE



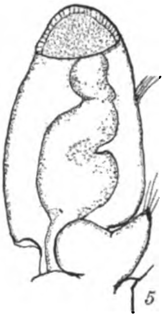
1 a



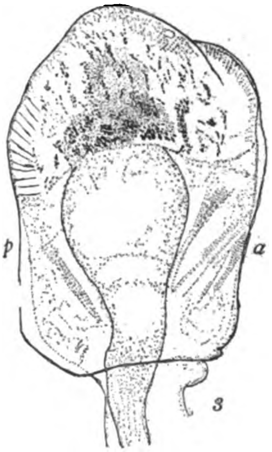
1 b



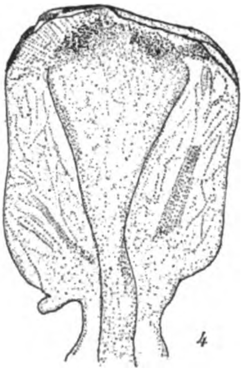
2



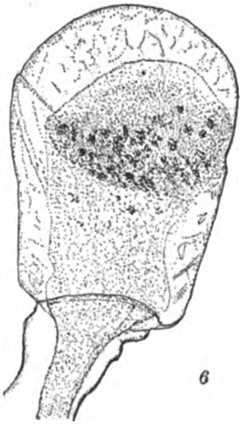
5



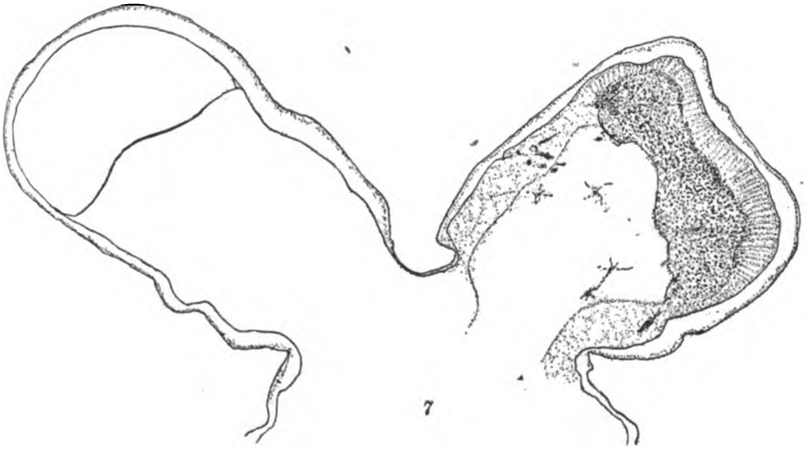
3



4



6



7

PLATE II

Fig. 8 Crangon, thirty-one days. One moult sixteen days after injury. Dorsal view. Operation removed small part of inner anterior ommatidial surface. Injured eye four-fifths length of normal. × 45.

Fig. 9 Palæmonetes, thirty-three days. First moult fourteen days after operation; second moult nine days later. Dorsal view. Injury chiefly on posterior ventral edge. Injured eye four-fifths length of normal eye. × 35.

Fig. 10 Palæmonetes, thirty-three days. First moult fourteen days after operation; second moult ten days later. Whole of ommatidial region destroyed. Regenerating eye four-fifths length of normal eye. × 35.

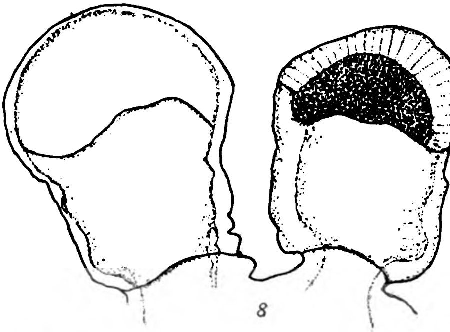
Fig. 11 Palæmonetes, thirty-five days. First moult sixteen days after operation. Ventral view. Part of ommatidia removed from ventral side. Upper end of eye more pointed than usual. Pigment appears unevenly distributed. Regenerated eye seven-eighths length of normal eye. × 45.

Fig. 12 Hermit crab, thirty-three days. First moult thirty-two days after operation. Dorsal view. Operation removed all of ommatidia and part of optic ganglion. Small, complete, new eye regenerated. New ommatidia shorter than normal. New eye two-thirds length of normal eye. × 45.

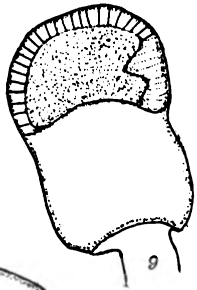
Fig. 13 Hermit crab, thirty-eight days. First moult twelve days after the operation. Dorsal view. Ommatidial region and nearly half of the ganglion removed. Very small but perfect eye regenerated. Regenerated eye four-sevenths length of normal eye. × 45.

REGENERATION IN COMPOUND EYES OF CRUSTACEA PLATE II

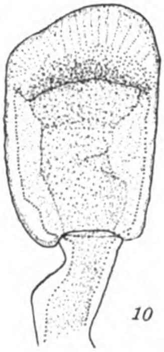
MARY ISABELLE STEELE



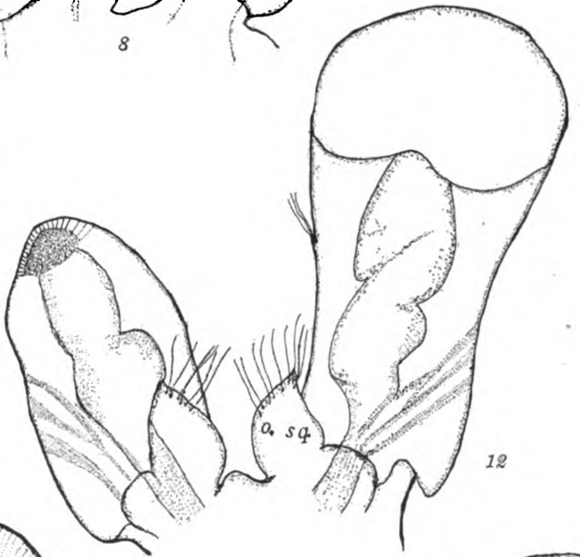
8



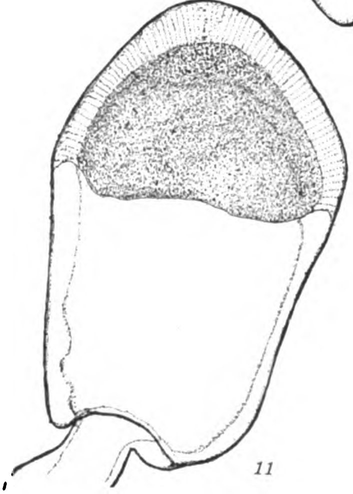
9



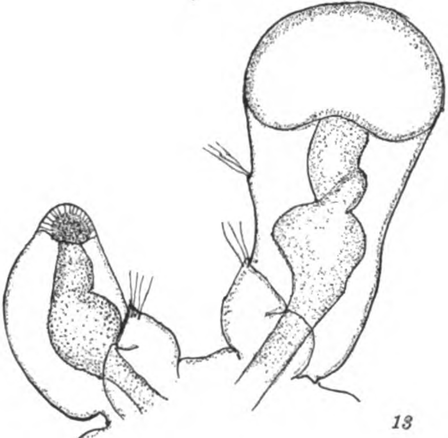
10



12



11



13

PLATE III

Fig. 14 Hermit crab, forty-one days. First moult forty-one days after operation. Dorsal view. Operation destroyed whole ommatidial portion and upper part of ganglion. Complete eye regenerated. Eye is about two-thirds length of normal eye. × 45.

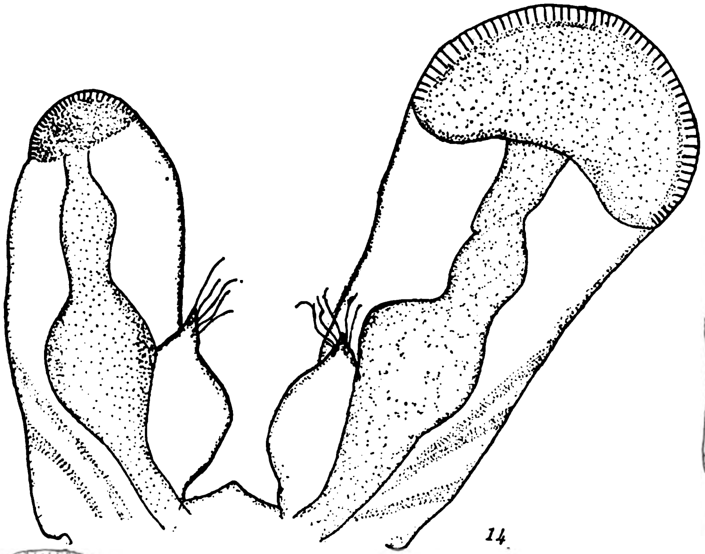
Fig. 15 Hermit crab, sixty-seven days. One moult forty-five days after operation. Whole ommatidial portion and upper part of ganglion destroyed. Regenerated eye fully differentiated. Regenerated eye two-thirds length of normal eye. × 45.

Fig. 16 Palæmonetes, sixty-five days. Moulted six days after operation. Eye split. New ommatidia regenerated. Regenerated eye six-sevenths length of normal eye. × 45.

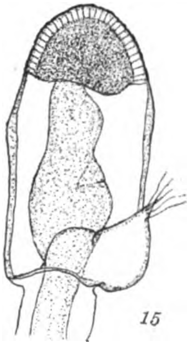
Fig. 17 Palæmonetes Viridis, nine days. One moult nine days after operation. Shows irregular development of upper end of eye. Pigment scattered irregularly. × 45.

Fig. 18 Ventral view of top of eye shown in Fig. 40. Regenerated material appears loose and reticular. × 90.

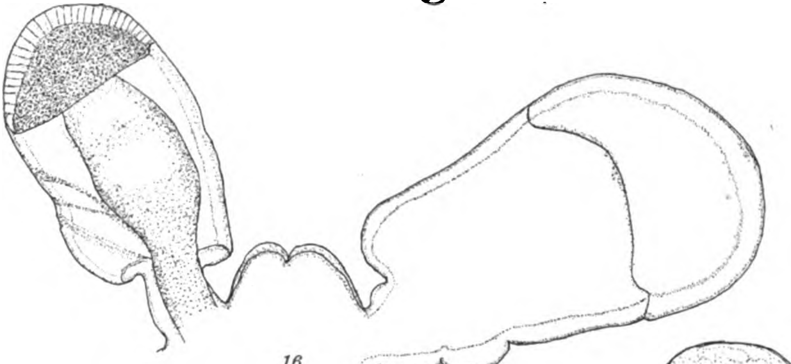
Fig. 19 Palæmonetes, thirty days. Dorsal view. First moult eight days after operation; second moult fifteen days later. Ommatidial portion wholly destroyed. Upper part of eye transparent. Regenerated tissue forms loose reticulum. No external signs of differentiation of ommatidia. Injured eye seven-eighths length of normal eye. × 45.



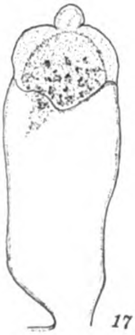
14



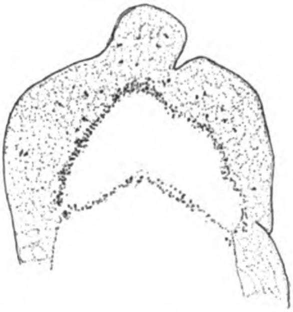
15



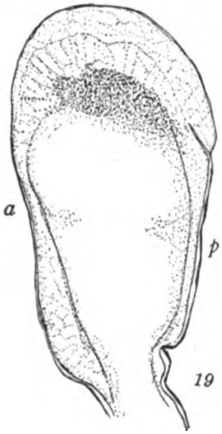
16



17



18



19

PLATE IV

Fig. 20 Palæmonetes, thirty-eight days. First moult ten days after operation; second moult fourteen days later. Ventral view. Entire ommatidial region destroyed. Dark band represents remains of old pigment. Regenerating eye five-eighths length of normal eye. × 45.

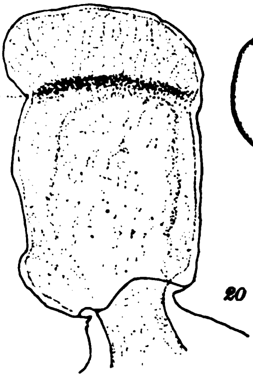
Fig. 21 Hermit crab, sixty-seven days. Moulted twelve days after operation. Stump shows large part of ganglion remaining but no signs of regenerating eye. Loose shreds of new tissue developed distal to the stump. × 45.

Fig. 22 Palæmonetes, seventeen days. Moulted seventeen days after operation. Eye split. Almost entire eye degenerated. Stump about one-third length of normal eye. × 90.

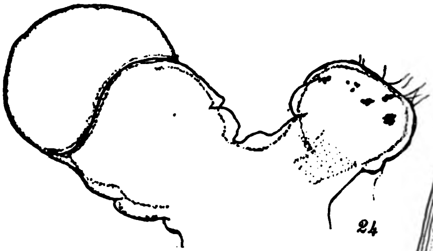
Fig. 23 Hermit crab, twenty-one days. Regenerated heteromorphic appendage and base of normal eye. One moult twenty-one days after operation. Appendage segmented; curves inward toward the median line. × 90.

Fig. 24 Eye stump, thirty-two days. First moult ten days after operation; second moult sixteen days later. Pigment patches near distal end of stump. Short stiff hairs on end of stump. Stump is two-fifths length of normal eye. × 45.

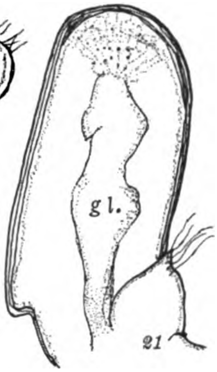
Fig. 25 Hermit crab. Injured when found. One moult. Ventral view. Distal end of eye shows regenerating ommatidia at *o*. Abnormal pigment developed at *pt*. Abnormal protuberance on the inner edge. Injured eye three-fifths length of normal eye. ×45.



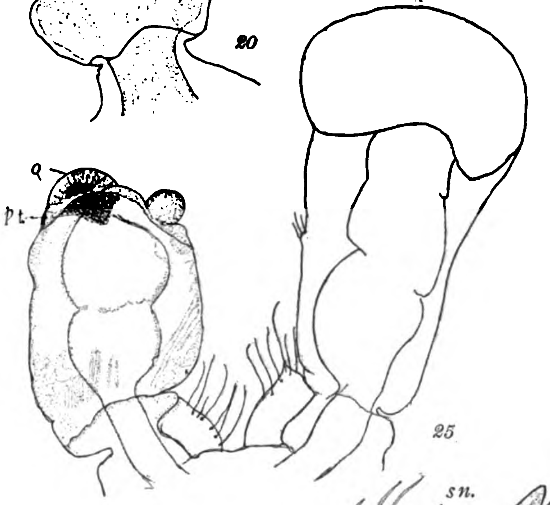
20



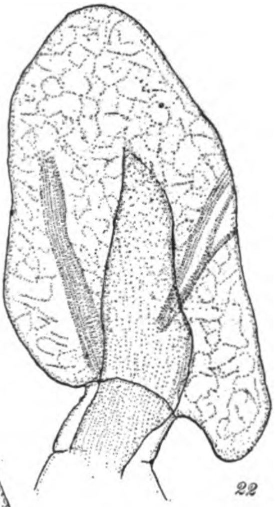
24



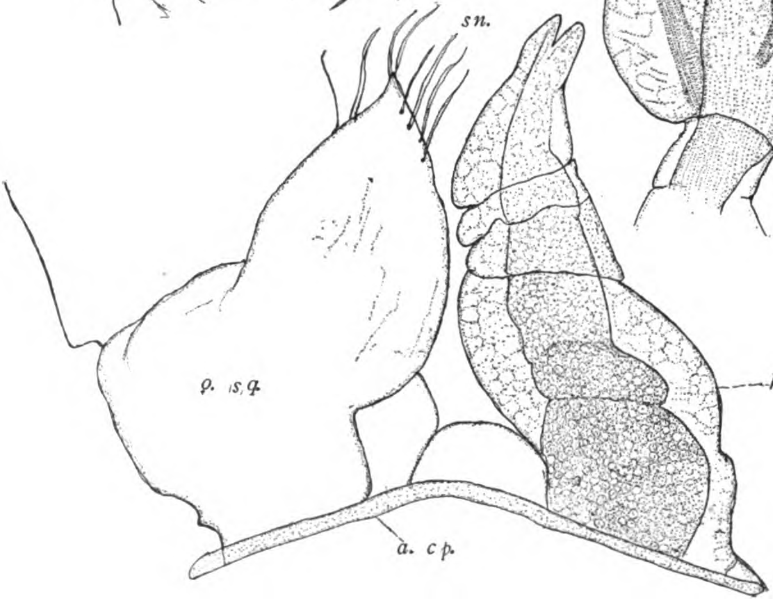
21



25



22



23

PLATE V

Fig. 26 Palæmonetes, twenty-four days. First moult ten days after operation; second moult fourteen days later. Right eye removed at level of line *a–b*. Small bud of new tissue. *t*, Regenerated from stump. Cuticle removed from stump. × 45.

Fig. 27 Stump shown in Fig. 26 more highly magnified. New tissue darkly shaded. × 125.

Fig. 28 Hermit crab, twenty-four days. One moult twenty-two days after removal of eye. Heteromorphic appendage. Ventral view. Cuticle heavy, appendage irregularly segmented. Sensory hairs developing near tip. Nerve trunk visible beyond proximal half of segment. Nerve trunk small in diameter as compared with Fig. 30. Appendage three-fourths length of normal eye. × 90.

Fig. 29 Palæmonetes, twenty-five days. One moult. Outline of normal eye and eye stump showing abnormal pigment. Pigment in a number of small masses on upper distal end of stump. × 75.

Fig. 30 Hermit crab, twenty-two days. Moulted twenty-two days after operation. Heteromorphic appendage. Dorsal view. Show segments, sensory hairs and nerve trunk extending beyond proximal half of appendage. Appendage one-third length of normal eye. × 125.

Fig. 31 Hermit crab, twenty-six days. One moult twenty-six days after operation. Dorsal view of normal eye and healed over stump. Stump three-eighths length of normal eye. × 45.

REGENERATION IN COMPOUND EYES OF CRUSTACEA PLATE V

MARY ISABELLE STEELE

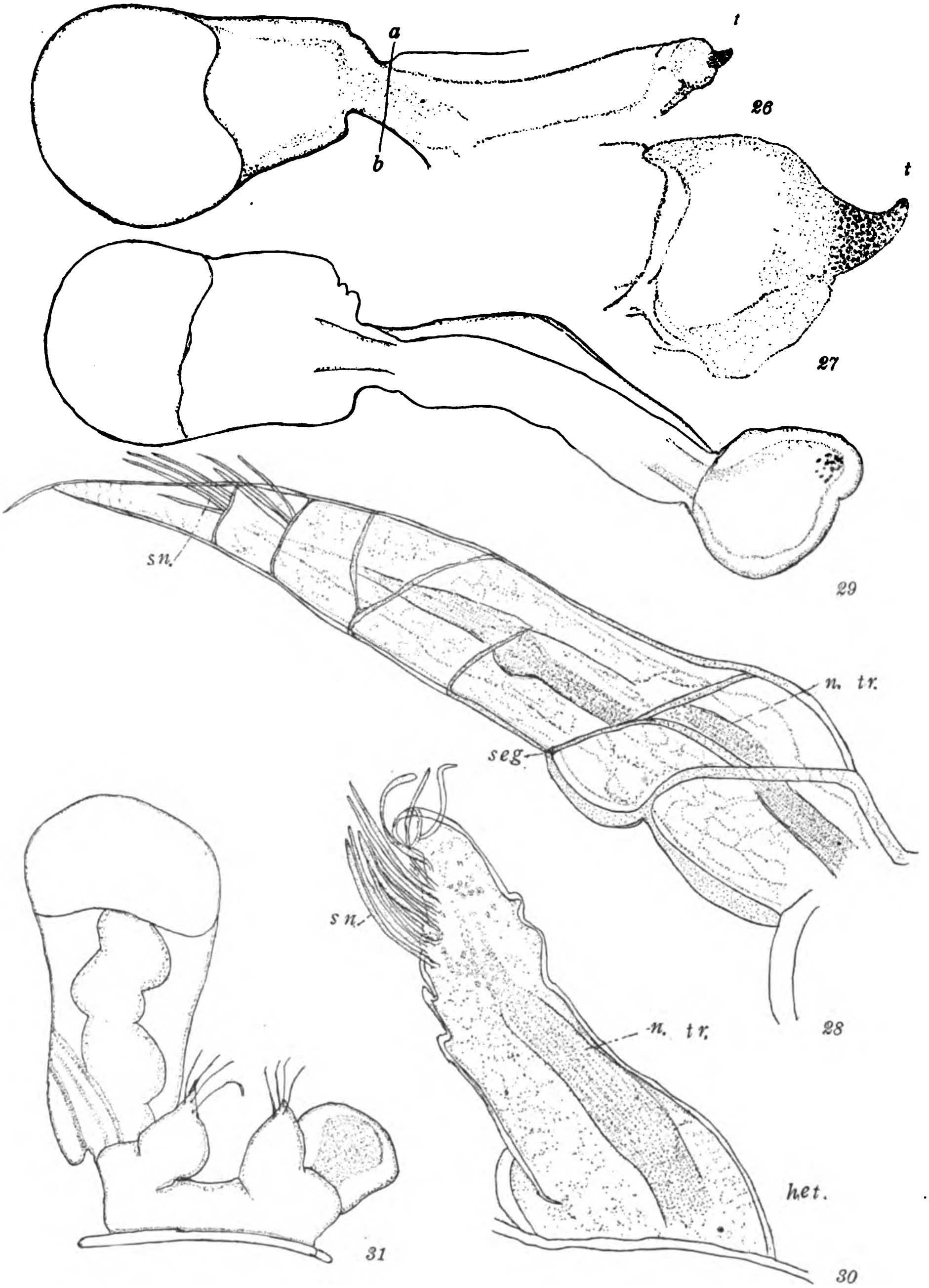


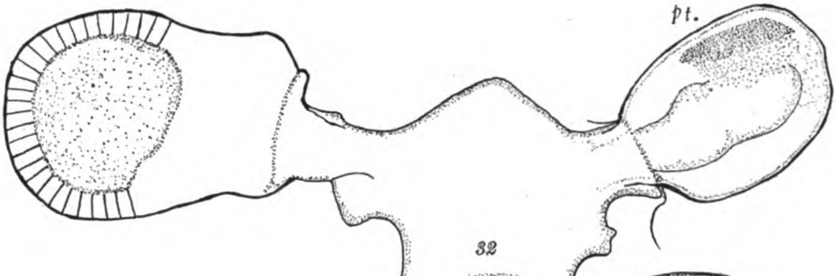
PLATE VI

Fig. 32 Palæmonetes, twenty-seven days. First moult sixteen days after operation; second moult ten days later. Outline of normal eye and eye stump showing abnormal pigment. Pigment appears as granular area on inner dorsal surface. Eye stump four-fifths length of normal eye. × 45.

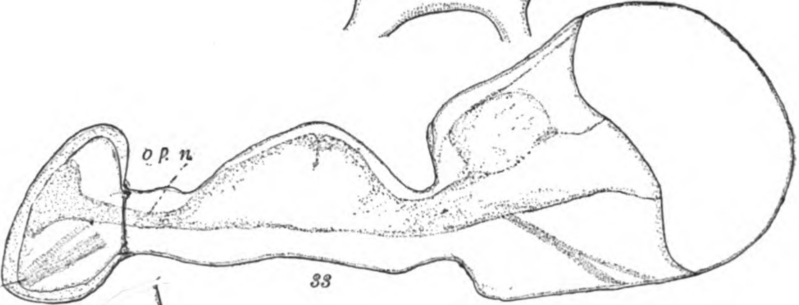
Fig. 33 Palæmonetes, twenty-seven days. First moult ten days after operation; second moult fourteen days later. Ventral view of stump and normal eye. End of optic nerve stump flush against the cuticle. Optic nerve reduced in size, two-sevenths lengths of normal eye. × 45.

Fig. 34 Hermit crab, thirty-nine days. Moulted twenty-nine days after operation. Dorsal view of normal eye and heteromorphic appendage. Shows optic squame in connection with appendage. Optic nerve stump extends through proximal half of appendage. × 35.

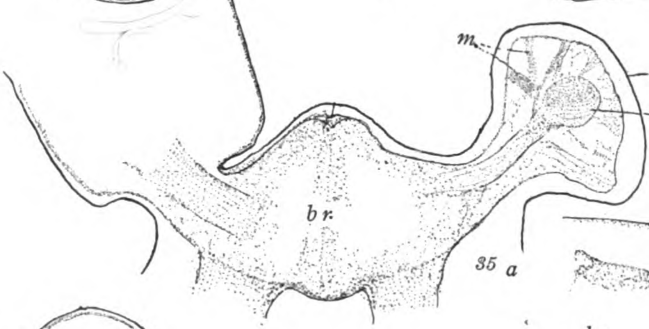
Fig. 35 Series of Palæmonetes eye stumps after removal of greater part of eye. *a*, Eye stump with small quantity of new tissue developed beyond end of optic nerve stump. Stump measures one-third length of normal eye. × 45. *b*, Eye stump that shows no regeneration. Twenty-nine days. Moulted ten days after operation. One-fourth length of normal eye. × 45. *c*, Eye stump showing an unusual development of new tissue. Moulted ten days after operation. Stump two-sevenths length of normal eye. × 45.



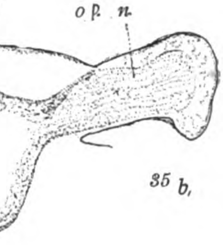
32



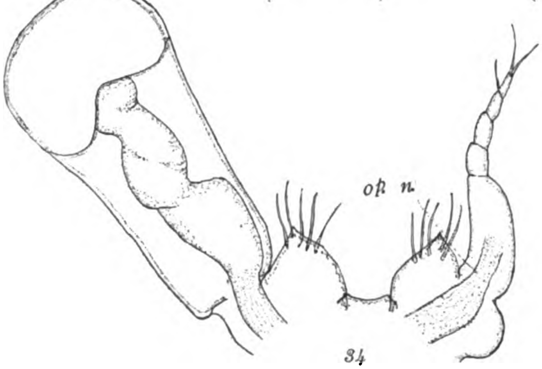
33



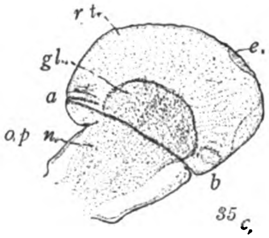
35 a



35 b,



34



35 c,

Plate VII

Fig. 36 *a* and *b*, Palæmonetes, thirty days. One moult. Optical section of normal and regenerating eye. Ventral view. *a*, Shows regenerating eye; *b*, normal eye for comparison. Operation apparently removed eye near level of line *a–b* on normal eye. Regenerating eye shows considerable new tissue and pigment spot on ventral side. Heavy cuticle over end of stump. × 45.

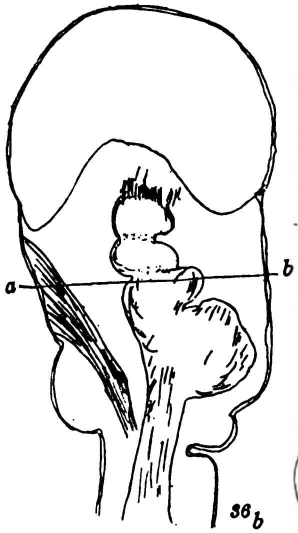
Fig. 37 Crangon eye stump, thirty-two days. First moult two days after operation; second moult nine days later; third moult sixteen days later. Stump one-third length of normal eye. No regeneration. × 45.

Fig. 38 Crangon, thirty-two days. First moult four days after operation; second moult seventeen days later. *a*, Optical section of heteromorphic appendage and outline of normal eye. Dorsal view. × 45. *b*, Ventral view of heteromorphic appendage more highly magnified. Shows six segments and sensory hairs developed on the inner distal edge. Nerve trunk apparent through greater part of length. Appendage measures four-fifths length of normal eye. × 90.

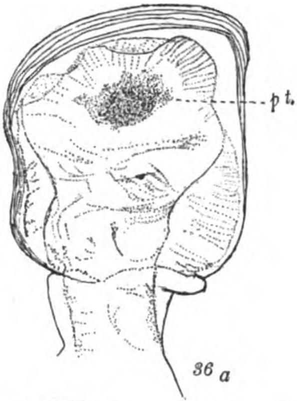
Fig. 39 Hermit crab, thirty-two days. One moult thirty-two days after operation. Ventral view of stump showing no regeneration. Stump two-thirds length of normal eye. × 45.

Fig. 40 Crangon eye stump, thirty-one days. First moult seven days after operation; second moult twelve days later. Stump measures one-third length of normal eye. × 45.

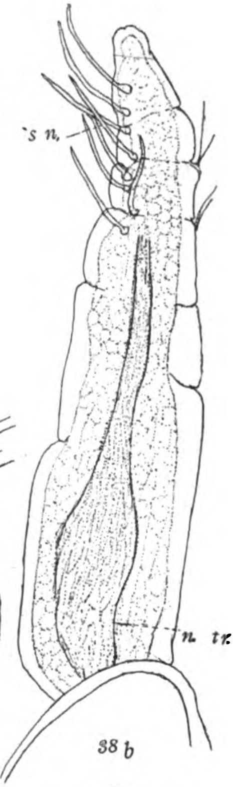
REGENERATION IN COMPOUND EYES OF CRUSTACEA PLATE VII
MARY ISABELLE STEELE



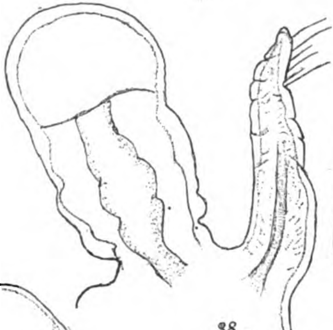
36 b

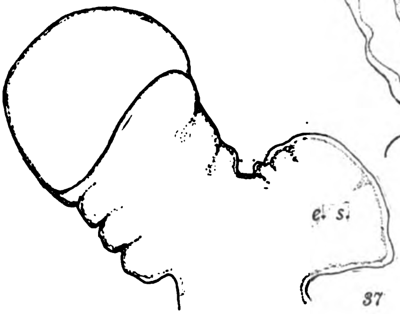


36 a

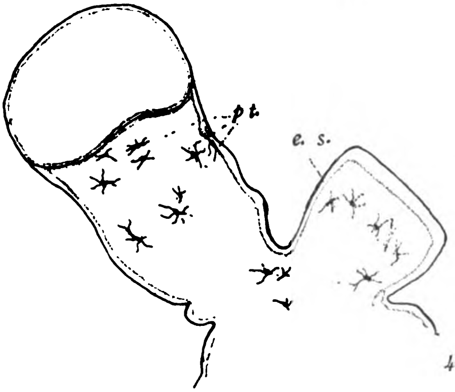


38 b

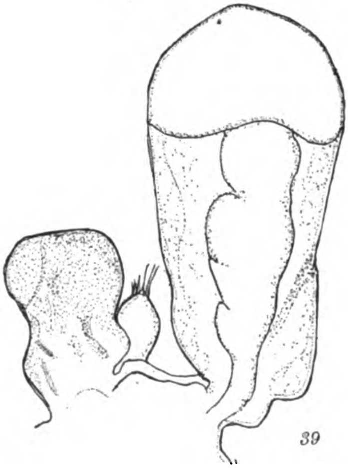

38 a



37



40


39

THE JOURNAL OF EXPERIMENTAL ZOÖLOGY, VOL. V, NO. 2

PLATE VIII

Fig. 41 Hermit crab, thirty-two days. One moult thirty-two days after operation. Dorsal view of normal eye and heteromorphic appendage. Appendage small and sharply curved backward. Shows several segments and a few sensory hairs. × 90.

Fig. 42 Crangon eye stump, thirty-two days. First moult eight days after operation; second moult twelve days later. Cuticle folded and wrinkled. Short hairs on end of stump. Stump about one-half length of normal eye. No regeneration. × 45.

Fig. 43 Palæmonetes, thirty-eight days. Two moults. Eye stump and outline of normal eye. Ventral view. Shows abnormal pigment spot. Eye stump one-half length of normal eye. × 45.

Fig. 44 Palæmonetes, thirty-eight days. First moult sixteen days after operation; second moult eighteen days later. Eye stump showing abnormal pigment which appears as a single solid mass on upper anterior border of eye stump. Stump about two-thirds length of normal eye. × 45.

Fig. 45 Hermit crab, thirty-nine days. One moult. Heteromorphic appendage. Dorsal view. Nerve trunk distinct in proximal part of appendage. Appendage three-fifths length of normal eye. × 90.

MARY ISABELLE STEELE

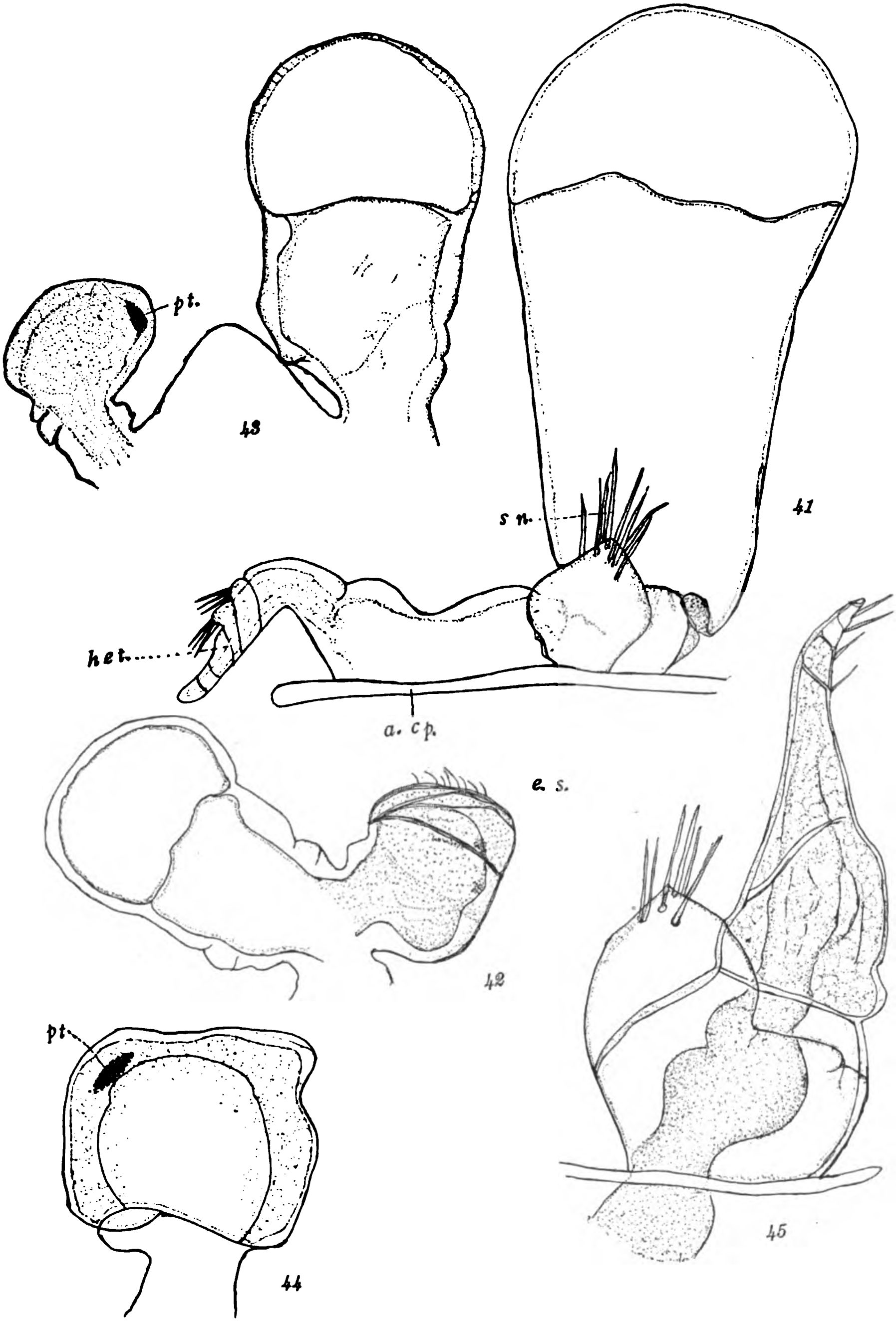


PLATE IX

Fig. 46 Semidiagrammatic section through the top of eye of Cambarus virilis sixty-two and one-half hours after removal of part of ommatidia. Shows relation of cuticle and protective crust (*cr.*) Shows broken down tissue excluded by crust also. Below crust is space from which inner tissues have shrunk. Space occupied by coagulated plasma. × 90.

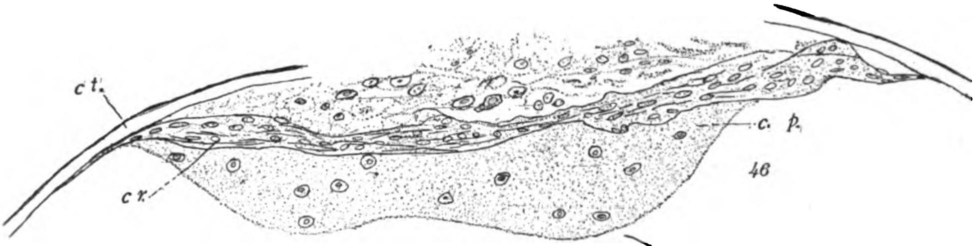
Fig. 47 Semidiagrammatic section through upper part of eye of Cambarus gracilis showing contiguity between regenerated and old cuticle. Also shows broken down tissues excluded by development of cuticle. Eye operated upon by tearing small hole in cornea with needle. × 450.

Fig. 48 Section from eye shown in Fig. 9. Broad band of new cuticle developed. Few regenerated nuclei present. All the tissue shown below cuticle degenerating remains of old ommatidia. × 1350.

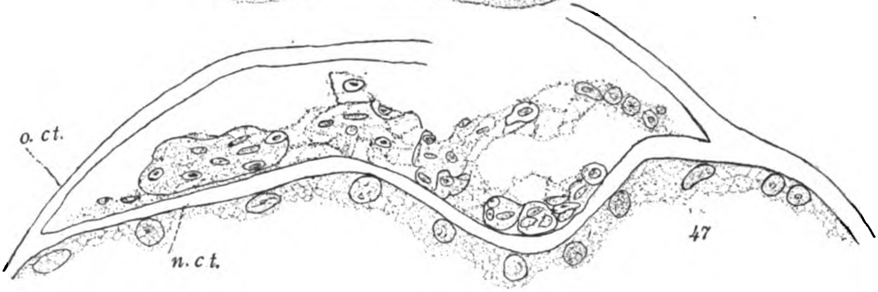
Fig. 49 Section from eye shown in Fig. 3. Shows new cuticle with no hypodermal cells beneath it. Shows amitotically dividing nuclei. × 1350.

Fig. 50 Part of section from eye shown in Fig. 3. Section from near edge injured area. Hypodermal nuclei much more numerous than in Fig. 51, which is taken from a section near center of injured area. × 600.

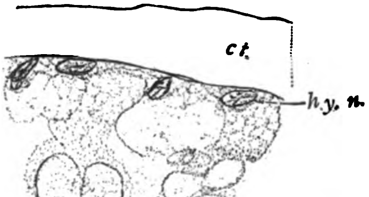
Fig. 51 Section from eye shown in Fig. 3. New cuticle but no well defined hypodermis yet formed beneath cuticle. Granular masses and pigment patches are remains of degenerating ommatidia. × 430.



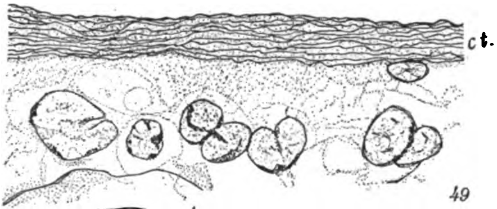
46



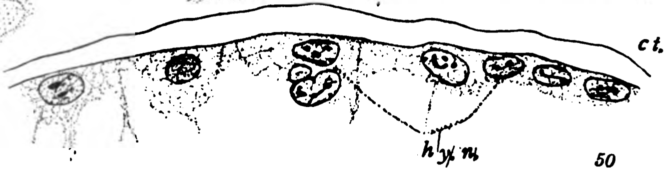
47



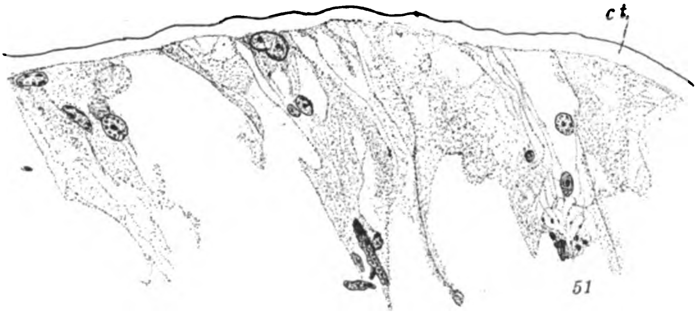
48



49



50



51

PLATE X

Fig. 52 *a*, *b*, *c*, *d* and *e* shows series of figures from an eye of Palæmonetes representing transformation of corneal hypodermal cells into active regenerating hypodermis. Shows normal corneal hypodermal cells together with corneal facets and tops of cones in *a* and *b*. Transformation of cells from resting corneal hypodermal cells to active regenerating cells in *c* and *d*. These form a continuous series. × 1350.

Fig. 53 Section of eye of Palæmonetes from which part of the ommatidial region was removed. Shows new cuticle and reticular subcuticle. Transformed hypodermal cells in process of amitotic division. To the left a single cell which may be undergoing mitotic division. Experiment covered twenty-three days. Moulted twice. Section taken from same eye as series in Fig. 52. × 1350.

Fig. 54 Sections from same eye as Figs. 52 and 53. This section shows cells separating from the hypodermis and also early stages of differentiation of retinulæ. Outline of cuticle and subcuticle shown. × 660.

Fig. 55 Section from eye shown in Fig. 10. Hypodermal cells differentiated. Amitosis taking place in these and the deeper lying cells. The deeper cells are regenerating retinular cells. × 1350.

MARY ISABELLE STEELE

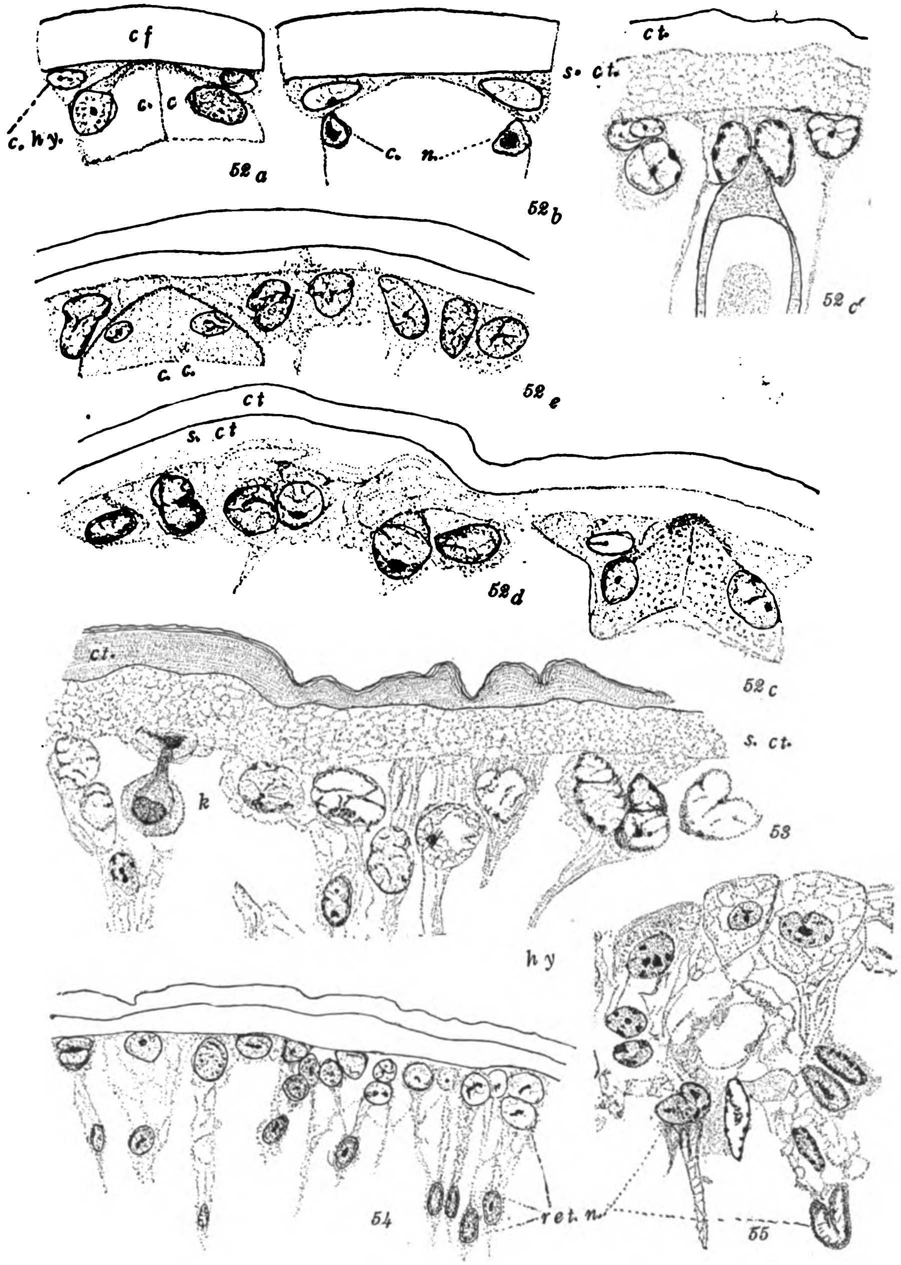


Plate XI

Figs. 56–59 Taken from sections of regenerating eye shown in Fig. 36. Fig. 56 from section near dorsal surface. Regenerated tissue lies for the most part peripheral to the broken line *x–y*. Cuticle torn and inner tissues shrunken from it. Old tissues show parts of muscle bands and small groups of ganglion cells. Distal to muscle band new tissue seems differentiated into fibers. × 90.

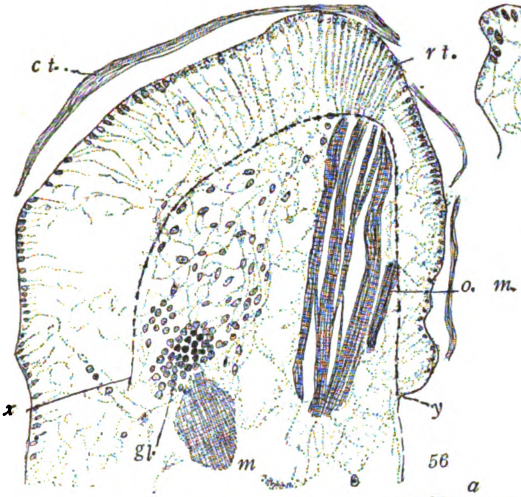
Fig. 57 Shows section deeper below surface than Fig. 35. Same features as in preceding figure. In addition a few small pigment masses. An increase in size of the nuclei in region from *b* to *c*. Cuticle not shown. × 90.

Fig. 58 Represents upper part of tangental section near the ventral surface. Nuclei increased in size and number over those in preceding figure. Rudimentary ommatidial elements apparent in new tissue. Figure composed entirely of regenerated tissue except small group of ganglion cells. × 90.

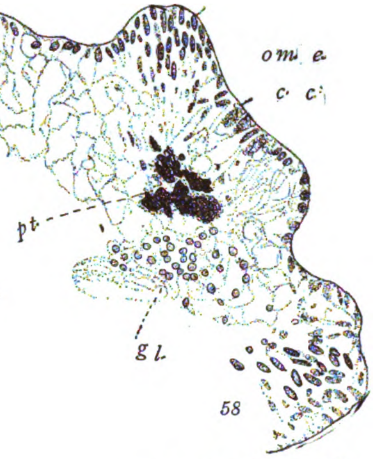
Fig. 59 Rudimentary ommatidia from the eye shown in Fig. 36. Sections oblique so that entire ommatidium cannot be recognized. Shows distal ends of cones, retinular nuclei and pigmented processes which appear to be retinulæ. × 900.

REGENERATION IN COMPOUND EYES OF CRUSTACEA PLATE XI

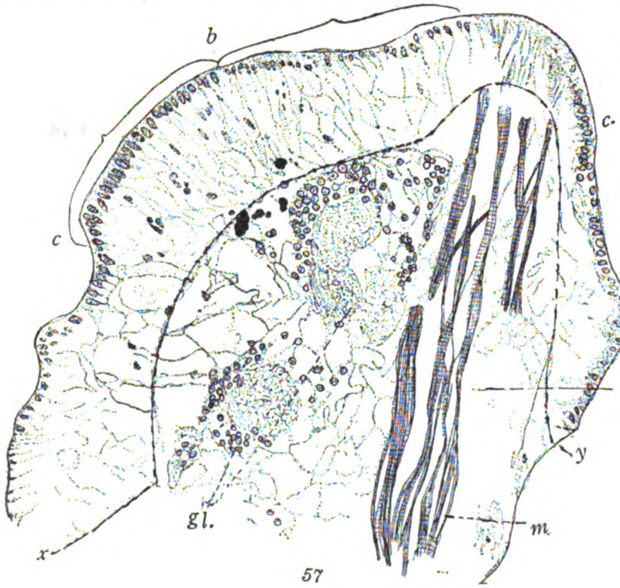
MARY ISABELLE STEELE



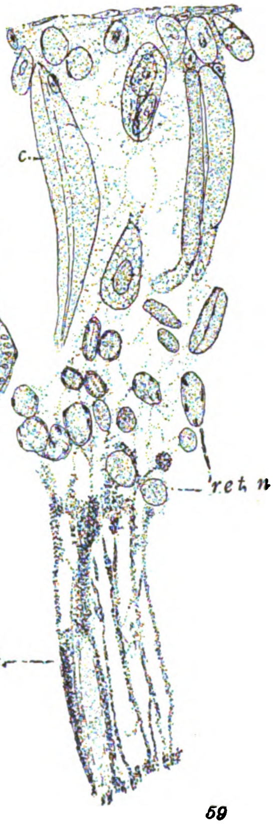
56



58



57



59

PLATE XII

Fig. 60 Group of retinulæ from eye shown in Fig. 6. Proximal retinular processes are seen extending to basement membrane. Two of the processes can be traced through below the basement membrane. × 900.

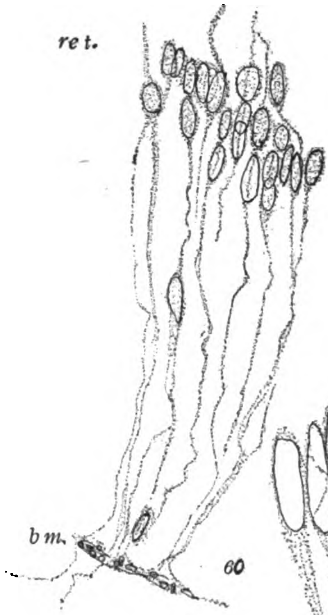
Fig. 61 Taken from section of eye shown in Fig. 16. Shows group of retinulæ. Nuclei in outline and proximal processes shown. × 1350.

Fig. 62 Group of retinulæ from eye shown in Fig. 6. Shows proximal retinular processes penetrating basement membrane and twining among ganglion cells below. × 1350.

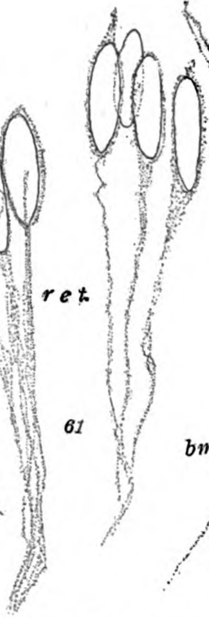
Fig. 63 Taken from section of eye shown in Fig. 11. Shows early stage in differentiation of crystalline cones. Cone nuclei are being separated from hypodermal nuclei. Hypodermal nuclei are grouped in pairs. Delicate strands of cytoplasm extending inward from pairs of nuclei. × 1350.

Fig. 64 Taken from section of eye shown in Fig. 11. Shows more advanced stage of cone differentiation than Fig. 29. Cell outlines becoming defined but hypodermal and cone cells not distinctly separated. Section somewhat oblique so that the four cone nuclei are visible. Distal retinular processes extending between the cones. Lower ends of cones not yet differentiated. × 900.

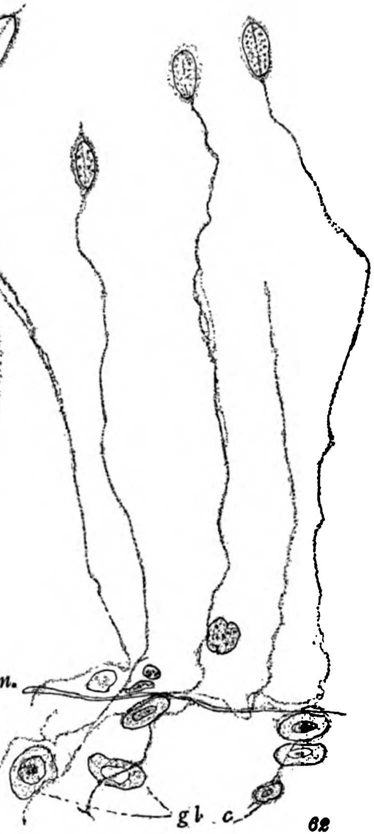
MARY ISABELLE STEELE



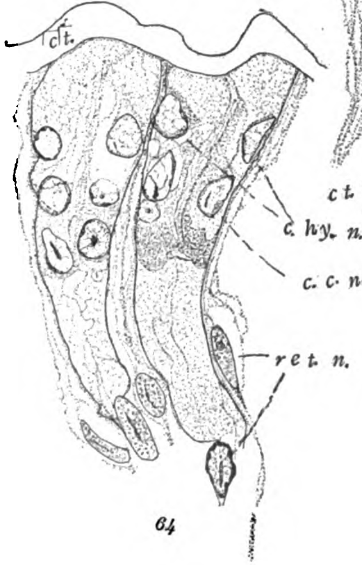
60



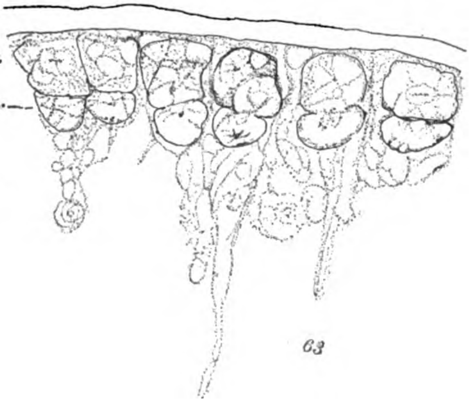
61



62



64



63

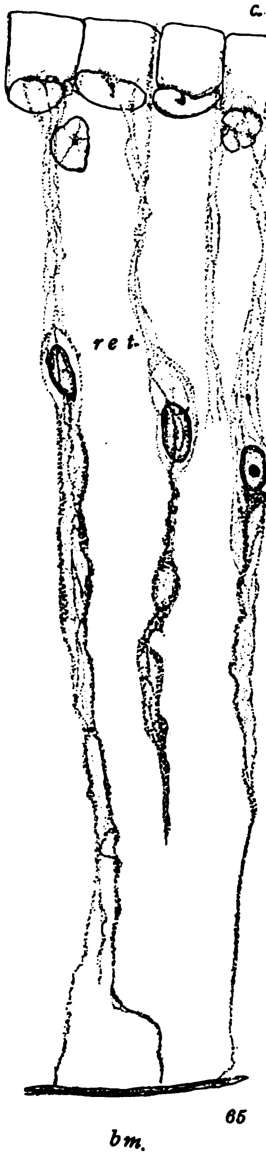
Plate XIII

Fig. 65 Taken from section of eye shown in Fig. 11. Shows retinulæ with their distal processes extending to hypodermis. Shows early pigment deposition in proximal processes. Hypodermal cells shown in outline. × 1350.

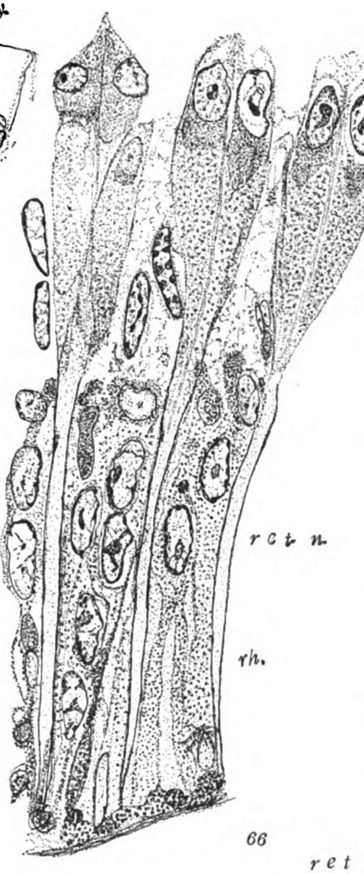
Fig. 66 Taken from section of eye shown in Fig. 11. Ommatidia completely differentiated except spindle shaped enlargement of the rhabdom. Distal ends of cones not yet differentiated completely. Cone at left of figure cut obliquely. Retinulæ not altogether normal in their distribution. × 1350.

Fig. 67 Regenerated ommatidium from eye. Shown in Fig. 11. Rhabdom still not quite normal in appearance. × 1350.

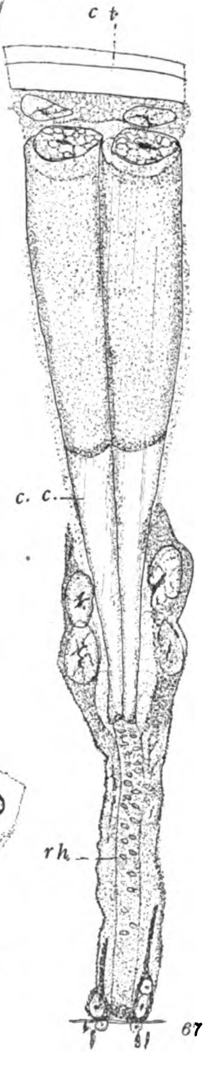
Fig. 68 Sections from an eye shown in Fig. 15. Shows differentiated hypodermis and retinular nuclei beginning to assume their definitive position. Hypodermis and retinulæ both show dividing nuclei. × 1350.



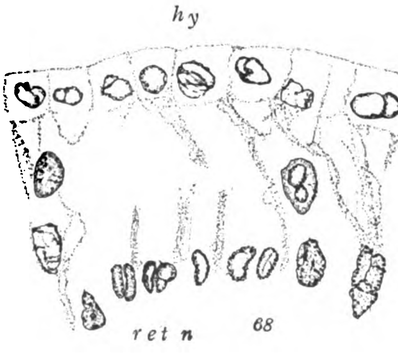
65



66



67



68

PLATE XIV

Fig. 69 Regenerating cones from eye shown in Fig. 20. Most of regenerated part of the eye occupied by abnormal tissue. Abnormal cells mingled with the normally regenerating structures. Compare Figs. 68–71. × 600.

Fig. 70 Part of section of eye shown in Fig. 20. Most of the cells abnormal polymorphic nucleate cells except those comprising the hypodermis. × 450.

Fig. 71 Right hand edge of Fig. 70 more highly magnified. Shows dividing hypodermal cells at upper edge and cells with polymorphic nuclei in interior. Three retinular nuclei at right edge of figure. × 1350.

Fig. 72 Outline of section through stump shown in Fig. 43. Shows location of pigment spot with reference to other structures in stump. × 125.

MARY ISABELLE STEELE

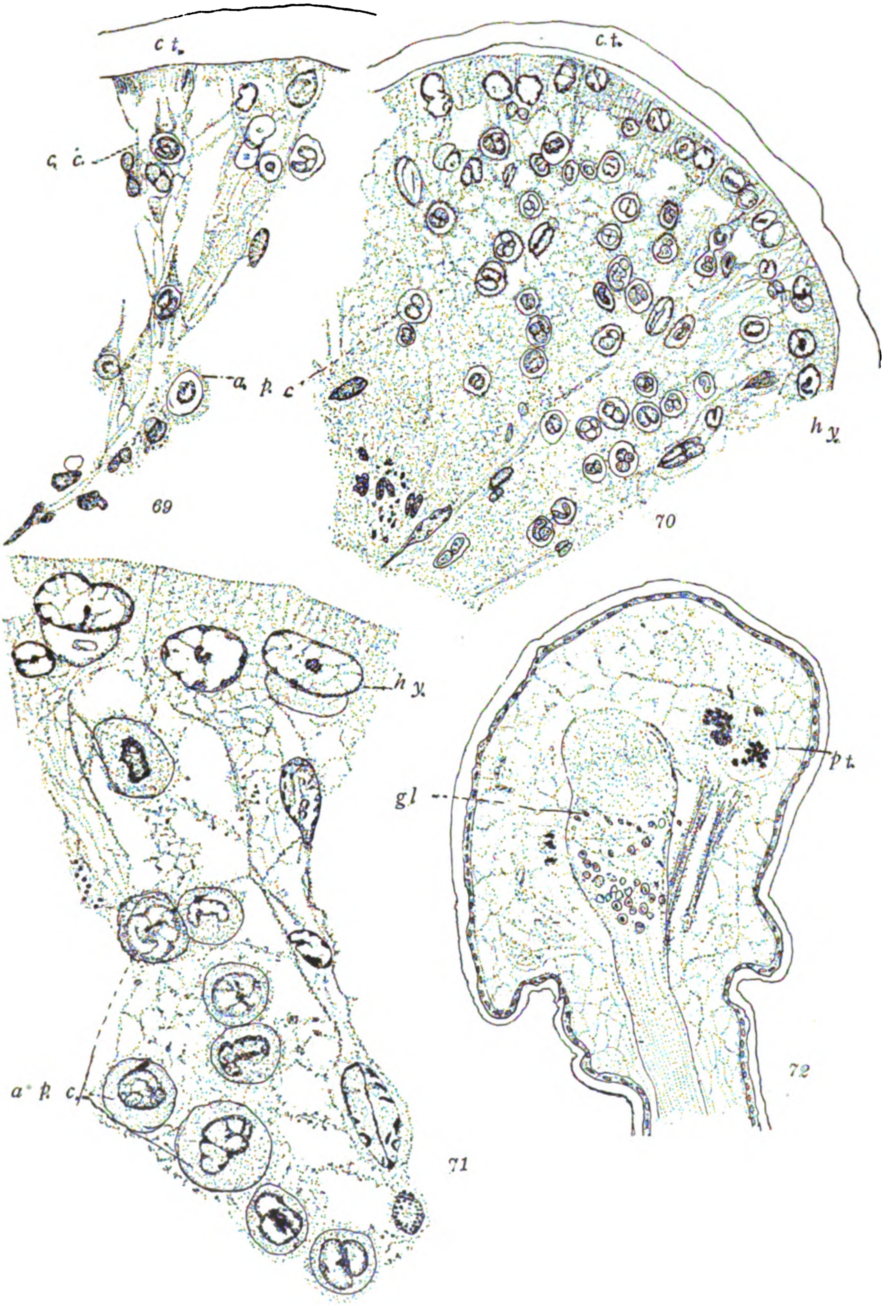


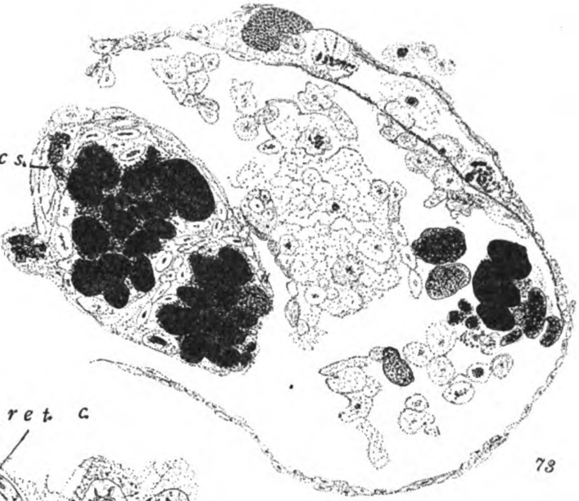
PLATE XV

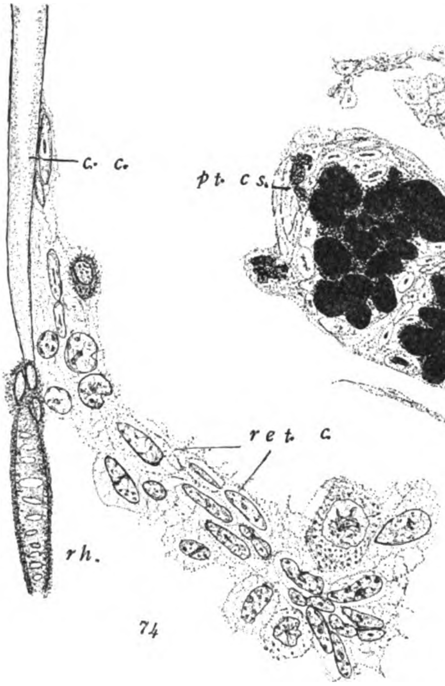
Fig. 73 Detailed representation of pigment area shown in preceding figure. Shows collection of pigment cells within cysts. × 900.

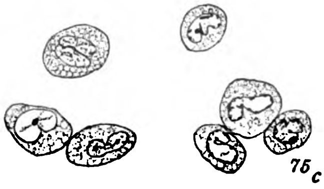
Fig. 74 Part of section from Cambarus virilis sixty-two and one-half hours after operation. In left part of figure lower part of cone and upper end of rhabdom. Remainder of figure occupied by chains of abnormal cells apparently developing from disintegrating retinulæ. A few cells show pigment granules. × 910.

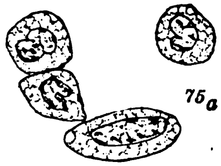
Fig. 75 Group of cells which show polymorphic nuclei. *a*, Group of disintegrating retinulæ from Cambarus virilis seventeen and one-half hours after operation; *b*, group of disintegrating retinulæ from Cambarus virilis thirty-nine hours after operation; *c*, disintegrating retinulæ from Cambarus gracilis sixteen days after operation; *d*, group of abnormal pigment cells from Crangon twenty-three days after operation; *e*, group of abnormal pigment cells from hermit crab sixty-seven days after operation; *f*, group of depigmented pigment cells from pigment cyst in eye of stump of Palæmonetes thirty days after operation; *g*, outline of crushed pigment body lying in same group with depigmented cells shown in *f*. × 900.

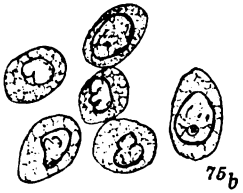
Mary Isabelle Steele

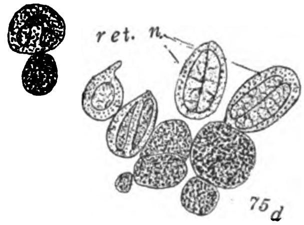


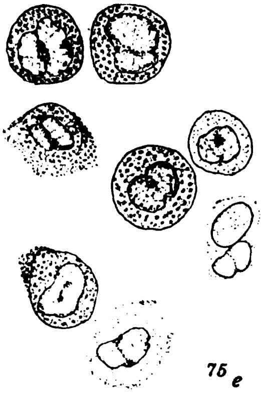


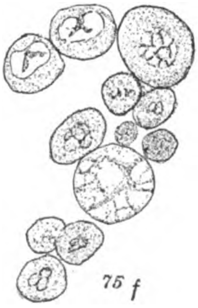












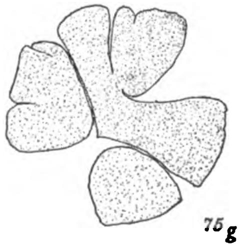


PLATE XVI

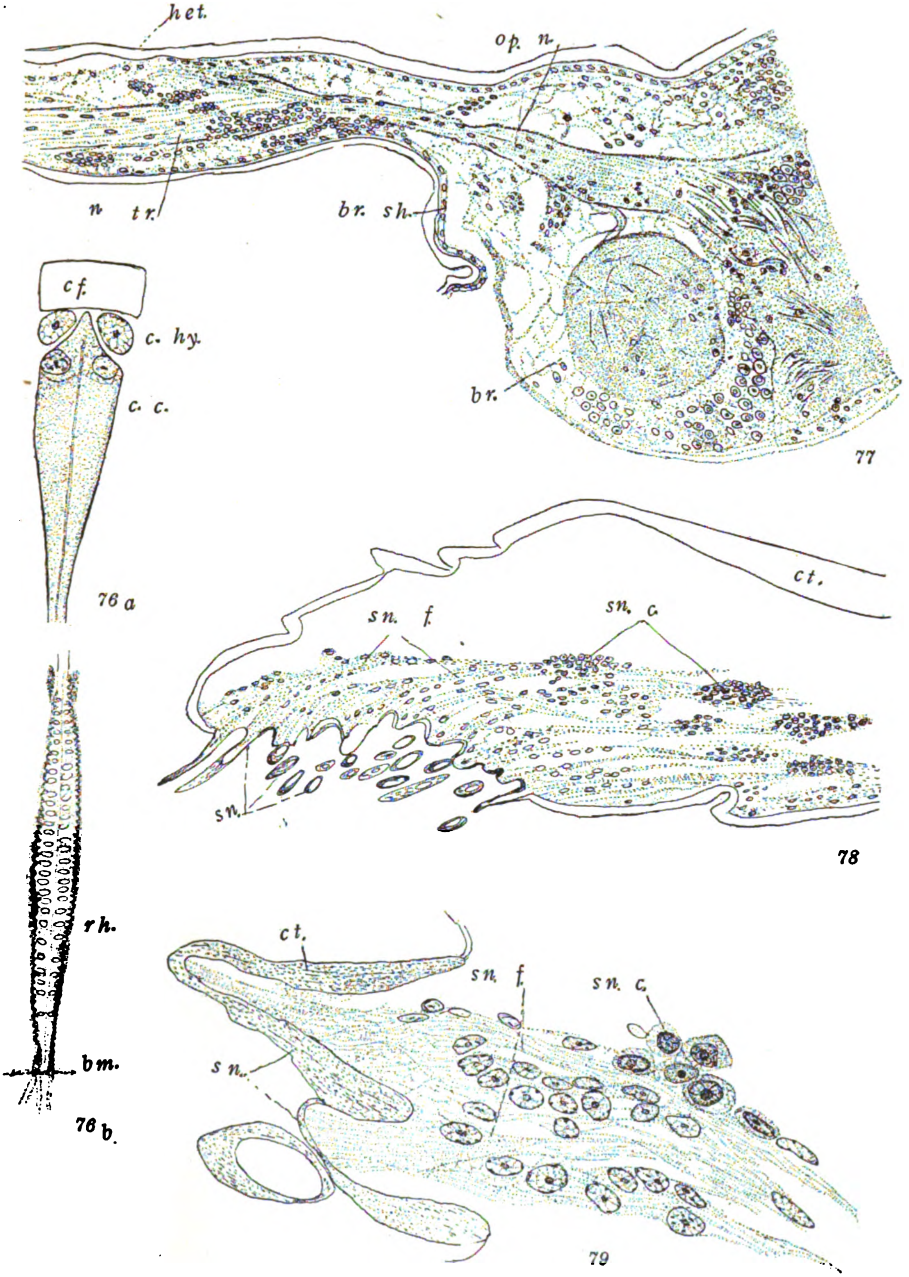
Fig. 76 *a*, Corneal facet and upper end of cone from fully regenerated ommatidium; *b*, fully regenerated rhabdom *a* and *b* both taken from regenerated hermit crab eye shown in Fig. 15. Ommatidia of hermit crabs much more slender than Palæmonetes ommatidia. × 900.

Fig. 77 Hermit crab, sixty-seven days. Section through brain and proximal end of heteromorphic appendage. Shows continuity of optic nerve and nerve trunk of appendage. Slightly diagrammatic. × 125.

Fig. 78 Section through distal end of heteromorphic appendage, showing strands of fibers *sn.f.* extending to the sensory hairs, and groups of sensory cells, *sn.c.* × 125.

Fig. 79 Detail drawing of small part of section shown in Fig. 78. Shows sensory cells and fibers *sn.f.* in connection with bases of two sensory hairs. × 750.

MARY ISABELLE STEELE



[Reprinted from BIOLOGICAL BULLETIN, Vol. XI., No. 4, September, 1906.]

THE FORMATION OF NEW COLONIES OF THE ROTIFER, MEGALOTROCHA ALBOFLAVICANS, EHR.

FRANK M. SURFACE.

Colony formation is not common among the Rotifera. But in several species, belonging especially to the family Melicertidæ, the individuals do become aggregated into colonies. These animals do not reproduce by budding, as do so many colonial forms, nor in most cases does the colony contain the progeny of its members. Instead, the young are hatched as free swimming individuals which later become segregated into a separate fixed colony.

During the past winter, while a graduate student at the University of Pennsylvania, the writer made some observations on the formation of new colonies of *Megalotrocha alboflavicans*. Colonies of this large rotifer were found in abundance in the fresh-water tanks of the vivarium, whither they had been transferred some years before from the small pond in the botanical gardens. These colonies are ordinarily formed on the roots and stems of various water plants, but in the vivarium they were found only on the stems of *Myriophyllum*. The colonies are nearly spherical in shape and when adult may measure as much as 4 mm. in diameter. They are thus easily recognizable to the unaided eye, appearing as white spots on the dark or green stems. The colonies used were kept in glass jars in the laboratory and for observation a piece of the stem containing a colony was removed, placed in a small dish and observed with a Braus-Drüner binocular.

The work was undertaken at the suggestion of Professor E. G. Conklin, and it is a great pleasure to acknowledge my indebtedness to both him and Professor H. S. Jennings for their many valuable suggestions and kindly criticisms.

The colonies of *Megalotrocha* are not surrounded by gelatinous masses or tubes as are certain other species of the Melicertidæ.

The animals are however attached to the stem by a kind of mucilaginous substance secreted by a gland in the foot. This substance forms a thin layer on the stem over the area of attachment of the colony. The adult colonies usually contained eggs or at least soon produced them after being brought into the laboratory. The eggs are attached to the mother by means of an adhesive gland situated a short distance posterior to the opening of the cloaca. Usually only one or two eggs are found attached to a single individual, but sometimes as many as four were observed.

The method of egg deposition is interesting. The egg rapidly increases in size in the region of the vitellarium, and then passes slowly down the oviduct to the cloaca. When the egg with its large germinal vesicle has reached this region the animal bends towards the dorsal side in such a manner that the adhesive gland touches the protruding egg. The animal frequently remains in this position for some time, often bending still farther so that the corona points towards the foot. In this way the end of the egg is firmly pressed against the adhesive surface of the gland. The animal now slowly and by repeated attempts straightens itself and at the same time the egg is pulled from the cloaca and remains attached to the mother, where it undergoes development. One egg has scarcely been deposited before another can be seen enlarging in the region of the vitellarium. Eggs begin to form in all the individuals of a colony at approximately the same time. But an interval of three or four hours may elapse from the time the first egg is laid until all the animals have deposited eggs. During this time the individuals which deposited the first eggs have often deposited a second. This overlapping of broods is important, as will be pointed out later, in keeping the size of the colonies more nearly constant.

The length of time required for the young rotifers to hatch varies somewhat, depending on the temperature and other conditions. Usually they hatch in three or four days after deposition. The young rotifer when fully formed can be readily seen through the transparent egg membranes. For some hours before hatching frequent contractions of the body and movements of the cilia and mastax may be seen. By means of these contractions the young animal finally bursts the enveloping membrane and is able to swim about. The young rotifers possess an organization in most respects similar to the adult. But among the more important differences may be mentioned the following. The trochal disc is at this time no broader than the trunk, the whole animal tapering slightly towards the foot. At the foot there is a circlet of small cilia. On the anterior border of the trochal disc, near the dorsal side are two red eye spots which are lacking in the adult. The cement gland in the foot is proportionately larger in the young animal than in the adult.

The young rotifers are free swimming, but are always attached at the posterior end by a thread of adhesive material which they spin out much after the fashion of a spider. They swim about among the adult rotifers, sometimes venturing a short distance beyond the limits of the colony but always drawing back and continuing to move about among the old animals. During all this time they are attached to the old colony by the adhesive thread from the foot. By a continuation of this nervous crawling and swimming some of them finally get their webs so twisted together that they are brought into contact. Here they remain, apparently without secreting more of the adhesive thread. At first there are only two or three individuals thus approximated and these are near the center of the old colony. Soon other young rotifers get their webs entangled with that of the few aggregated ones and these are then added to the ball that is forming. Here as well as later, contact with the adhesive thread appears to act as a guiding stimulus to the young rotifers. As soon as a young animal comes in contact with the rather thick thread leading to the forming ball, it at once begins to move up or down this thread until it finally reaches the ball; this I have repeatedly seen. Usually the young animal does not at once attach itself to the ball, but continues to crawl about, often through the bunch of young rotifers. In this way it finally becomes attached to its comrades with its foot closely adhering to theirs. During all the time that the ball is forming, the individuals composing it act in a very nervous manner, constantly jerking, twisting, contracting and expanding in a most irregular way. By means of this continual twisting and squirming the forming ball succeeds in stretching the thread holding it fast, so that later it comes to lie outside the limits of the old colony (Fig. 1). In the course of time this thread breaks and the young ball swims away.

From the time the first individuals are hatched until the ball breaks loose there is usually a lapse of three or four hours. Since all the eggs of one brood are not deposited at the same time the period of hatching often extends over several hours. The long time that it takes for the ball to form and break away gives opportunity for the later individuals to hatch and get into the new colony. Often there are a few young that do not get into the ball before it breaks away. These swim about for a time near the old colony and then may settle down on a near-by stem. Apparently these do not long survive the attacks of their enemies, for later I have seldom been able to find them, and I have never seen an isolated adult. By thus losing a few individuals in every generation the colonies would evidently continue to get smaller, for after the ball breaks away no new individuals are added to the colony. Such a decrease in numbers would undoubtedly take place were it not for the overlapping of the broods. One generation of eggs is laid so soon after the other that, in many cases, a large number of the second brood hatch in time to get into the forming ball. Occasionally, but not often,

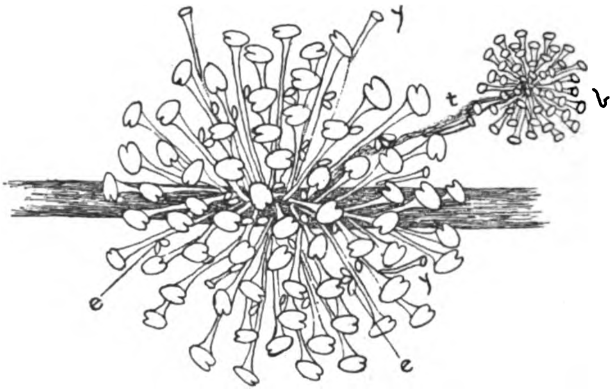


FIG. 1. Sketch to show formation of the swimming ball. *e*, eggs, attached to the adults; *y*, young animals before coming into the ball (*b*); *t*, mucilaginous thread holding the ball to the mother colony.

two balls are formed at the same time, thus dividing the young colony.

The ball of young individuals is spherical in shape and is very regularly formed. Indeed, this is one of the very striking things about these interesting balls. The feet of the individuals are not attached together in an irregular mass, as one might suppose, but they are arranged so as to form a very regular sphere. It is difficult to ascertain just how the animals are held together in these balls, but it seems probable that they are glued by the adhesive substance from the foot gland. This is supported by the fact that when a young ball is killed and placed in alcohol it breaks up, as if the adhesive material had been dissolved. The animals when in this ball are always nervously jerking back and forth. The ball swims freely by means of the currents from the trochal discs. If, for any reason, more of the individuals turn their trochal discs in a certain direction, the whole colony will move in that direction. That the movement of the ball is due to the summation of currents is shown by mixing some india ink with the water. It is then seen that the ball always moves in the direction from whence the stronger current comes. The ball swims with a revolving motion but is nevertheless able to move with considerable precision in a certain direction. A most important characteristic of these young balls is the fact that they react positively to light. One always finds them on the side of the dish next to the window. If they are placed in a dish in the sunlight, the balls congregate on the side towards the sun. If this region is shaded they *at once* leave it and move towards a part more strongly lighted. A considerable number of experiments showed that this reaction is very marked and constant.

The time which the animals spend in this swimming ball before settling down to form a permanent colony varies considerably, depending primarily upon the illumination. So far as I have observed, the colonies usually hatch out during the morning hours, the young ball breaking away about noon or afterwards. In no case have I seen them, under natural conditions, form a colony until evening or after sundown. As long as the dish is lighted, even by diffused light, the young rotifers continue to swim back and forth along the lighted side of the dish, striving apparently to get as near the source of light as possible. But if the ball is placed in artificial darkness they very soon begin to form a permanent colony.

The method of forming this permanent colony is interesting. When no longer influenced by the light the ball begins to move about the dish in an apparently aimless manner. If in this wandering it chances to come against some piece of water plant or other object many of the young rotifers turn their trochal discs towards this, thus checking the progress of the ball. This reaction is brought about by purely tactile stimuli. If a clean needle is placed in front of a swimming ball, the latter will stop and the young rotifers move their trochal discs along the needle. Whether they will settle down and establish a colony seems to depend chiefly on food conditions. If the stem possesses but little débris the ball may move along it for some distance, but in most cases finally swims away. If, however, the stem has more débris attached to it the young rotifers persist in their efforts to place their trochal discs on this, probably in order to get the food particles. In this way the ball is prevented from moving away. If the ball remains in one place for a time, one or two of the rotifers will be seen dragging themselves out of the ball and moving slowly along the stem. In this manner they move a short distance and then jerk back, then start out again, each time going a little farther. Soon others come out of the ball and begin moving about, until a large number are found moving up and down the stem (Fig. 2). In moving along the plant the animals extend their bodies with the long axis parallel to that of the stem. A few of the cilia of the trochal disc and a few of those in the posterior circle appear to touch the stem and in this manner the animals "crawl" along the plant, reminding one very much of a brood of young caterpillars. But it is probable that the water currents are here as at other times the most effective agents of locomotion. In these movements there is no revolution on the long axis. In advancing along the stem the animal usually shows great hesitation in going over portions which have not been traversed by some member of the colony. In the space over which several rotifers have travelled others move with little hesitation, but on coming to new territory they move forward a short distance and then draw back, often repeating this several times. Thus each individual pushes some distance beyond the previous limit, but at last it usually turns around and moves back towards its fellows. This like certain observations previously mentioned seems to indicate that the adhesive thread which they always secrete as they move about, furnishes a guiding stimulus for the young rotifers. Sometimes an individual leaves the stem and swims a short distance from it, always attached by the thread.

By such movements the whole ball finally breaks up and the individuals are seen moving back and forth along the stem, sometimes venturing quite a distance from the majority of the colony. But soon a few individuals settle down at some point and remain fastened by their posterior ends, with the body projecting at right angles from the stem (Fig. 2, *c*). Soon other individuals attach themselves in a similar manner with their posterior ends close to those already attached. This is the beginning of the formation of the permanent colony. In a comparatively short time all of the young animals have attached themselves in a closely aggregated group surrounding or nearly surrounding the stem. This permanent colony may be formed close to where the ball broke

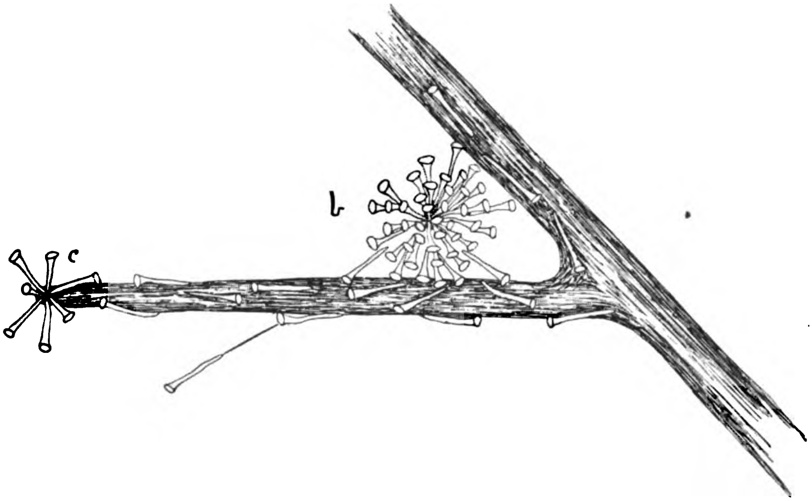


FIG. 2. Sketch showing the young ball (*b*) breaking up into free swimming individuals and the permanent colony forming at *c*.

up or at some distance from it. Sometimes it is formed at the end of a branch, sometimes at its base, or even on the main stalk; at other times in intermediate positions. If the swimming balls are allowed to remain in a dish containing no water plants soon after dark they begin to form a colony on the sides or bottom of the dish. Apparently when the stimulus from the light is removed the colony-forming instinct becomes dominant. When the young balls are placed in artificial darkness, colony-formation begins in from fifteen to thirty minutes and is usually completed within an hour or an hour and a half. Most of these colonies are found in well-lighted places. This doubtless arises from the fact that the swimming balls remain on the lighted side of the vessel and tend to form a permanent colony soon after this stimulus is removed. This is of advantage to the animals for they feed partly on diatoms and small protozoa which are more abundant in favorably lighted places.

Thus the young rotifers tend to remain attached in the swimming ball during their natatory life, but at the proper time they are able to sever this connection and to leave the ball one by one. Possibly the stimulus due to the contact of their posterior ends is sufficient to keep them together, until some stronger stimulus, such as food, overcomes the contact stimulus and induces them to separate. Hunger satisfied, they again respond to the stimulus of mutual contact and assemble anew, this time to form a permanent colony.

With the formation of this permanent colony changes take place both in the behavior and in the structure of the animals. Up to this time the young rotifers have behaved in a very nervous manner, constantly contracting and extending the body. But as soon as the animals settle down in the permanent colony they lose much of this irritability and remain with body and trochal disc expanded for long periods. Yet even in the adult colony one individual or another is frequently seen to suddenly fold the trochal disc and strongly contract the body, then at once begin to expand more slowly. Sometimes this contraction is evidently a reaction to some floating particle that has touched the trochal disc. More frequently there is no visible cause for the contraction. The animals in the permanent colony have another method of reacting, not found or at least not so well marked, in the free swimming balls. When the colony is stimulated as by a slight jar to the dish or a disturbance in the water the whole colony contracts at once and if the stimulus is strong they may remain contracted for some time. This is the usual method of reacting to mechanical, chemical or electrical stimulation.

After the young animals have once formed a permanent colony they become incapable of repeating the colony formation. If some of the individuals of a permanent colony are removed from the stem they do not behave as they did previous to the formation of the colony. They swim about by means of the ciliary currents, but in an entirely aimless fashion. They neither attach themselves to the old colony nor form a separate colony. I have kept such removed individuals alive for several days but during this time they made no attempt to reform a colony.

There are likewise several structural changes which take place after the formation of the permanent colony. The animals soon show considerable increase in size, the trochal disc becomes broader and the notch on the ventral side of this makes its appearance. The small circlet of cilia at the foot of the animal disappears and the character of the cement secreted by the foot-gland changes. This cement is no longer dissolved by alcohol as it was in the young specimens. The gland is always much smaller in the adult than in the free swimming individual. But the most striking change and one which perhaps accounts for some of the changes in behavior, is the degeneration of the eye spots. As stated before, the rotifers when hatched possess two red eye spots; the reaction to light of the swimming ball is probably due to these structures. After the permanent colony has been formed for several hours one finds that the eye spots have disappeared from their previous position while two small red bodies are floating about in the body cavity. In most cases these bodies are inclosed in floating corpuscles which have considerable resemblance to leucocytes. Montgomery ('03) mentions the presence of certain non-cellular corpuscles floating in the body cavities of certain Flosculariidæ. He regards these as waste products. Since in *Megalotrocha* these bodies often enclose the degenerating eye spots and these latter are seen to gradually waste away, it seems possible that they may function in the same manner as the leucocytes of many other animals. After the eye spots have disappeared from their original location the animals no longer react to light.

GENERAL AND COMPARATIVE.

The main features in the formation of new colonies of this rotifer are then as follows: When first hatched from the eggs the young are free swimming, but do not leave the colony singly. These individuals come together into a swimming ball which reacts positively to light. Later under certain conditions this breaks up into free individuals again. These then aggregate themselves into a permanent colony in which the animals spend the remainder of their lives. In this colony formation the mucus-like secretion of the foot-gland plays an essential part.

The details of colony formation in other rotifers has apparently not been described, but it seems probable from the known facts that a similar sequence of processes occurs in some other cases. In *Lacinularia socialis* according to Huxley ('53), Hudson and Gosse ('89) and others, the process appears to resemble that in *Megalotrocha*. The young animals come together into swarms or balls and swim freely. Later permanent colonies are found on water plants. In two species of *Megalotrocha* from China, *viz.*, *M. semibullata* and *M. spinosa* described by Thorpe ('89 and '93), the adult colonies are free swimming. In these cases the eye spots are present in the adults. The new colonies swarm out of the old free swimming ones. Occasionally these balls may be found suspended from aquatic plants by mucilaginous threads. In the genus *Conochilus* we find swimming colonies of a slightly different nature. Here the free swimming balls consist of several adults with many of their young. According to Hudson and Gosse many of the newly hatched rotifers make a place for themselves in the adult ball by squeezing between the older members, while others of the new brood form new balls and swim away. There are several other more or less rare species of Melicertidæ in which free swimming or fixed colonies have been described. The free swimming adult colonies are interesting as apparently marking a step in the formation of the fixed colonies. While the occurrence of colonies or swarms is common in the animal kingdom, I have seen no accounts of colony formation in other groups that resembles the processes seen in these rotifers.

BIOLOGICAL HALL, UNIVERSITY OF PENNSYLVANIA,
May 1, 1906.

LITERATURE CITED.

Huxley, T. H.

'53 Lacinularia socialis; a contribution to the Anatomy and Physiology of the Rotifera. Trans. of the Micros. Soc. of London, N. S., Vol. I., pp. 1–19.

Hudson and Gosse.

'89 The Rotifera. Two volumes, London.

Joliet, Lucien.

'83 Monographie des Melicertes. Arch. de Zool. Exp. et Gen., 2e Series, Tome I., pp. 131–224.

Montgomery, T. H.

'03 On the Morphology of the Rotatorian Family, Flosculariadæ. Proc. Acad. Nat. Sci. of Philadelphia.

Thorpe, V. Gunson.

'89 Description of a New Species of Megalotrocha. Journ. Royal Micros. Soc., pp. 1–4.

'93 The Rotifera of China. Journ. Royal Micros. Soc., pp. 145–152.

3 2044 106 245 210